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Dose effects on re-irradiation of the spinal cord

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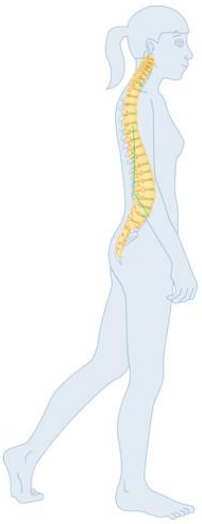

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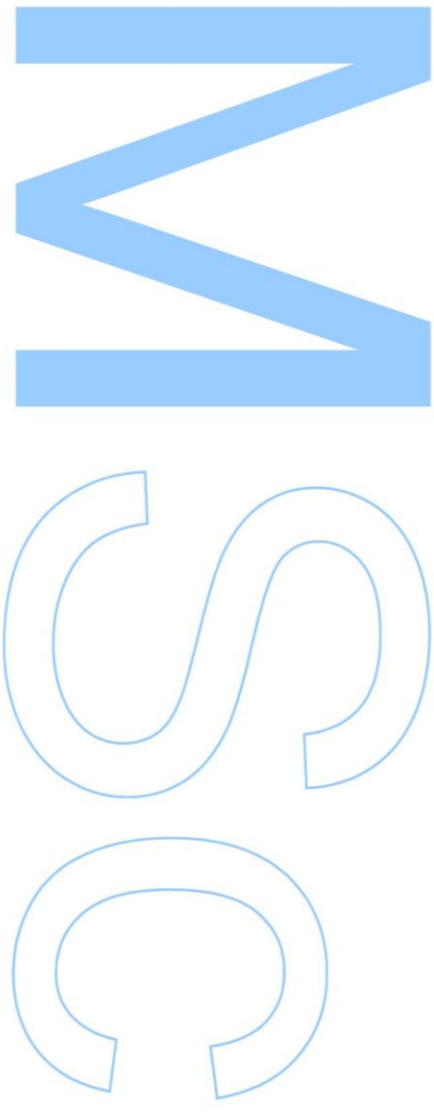
Todas as correções determinadas pelo júri, e só essas, foram efetuadas.
O Presidente do Júri,

Porto, _____/_____/_____

Declaração

Eu, *Helena Sofia Martins Alves*, nº 201602802, estudante do 2º ano do Mestrado em Física Médica no presente ano letivo 2017/2018, na Faculdade de Ciências da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração do texto apresentado, não apresento texto plagiado, e tomei conhecimento das consequências de uma situação de plágio.

Porto, 29 de outubro de 2018



Agradecimentos

Os meus agradecimentos são para as pessoas que me acompanharam e apoiaram ao longo do meu percurso escolar.

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Diego Vinicius

Abstract

Advances in biology and physics have allowed increased precision and accuracy in radiotherapy (RT) in order to maximize tumor damage and to minimize lesions in the dose limiting adjacent normal tissues. The spinal cord is the most critical organ at risk (OAR). Radiation myelopathy is one of the most devastating complications of clinical radiotherapy resulting in severe and irreversible morbidity. Assessment of the impact of dose and fractionation schemes on tissue tolerance has been a major area of research in radiation oncology. As a result of greater accuracy and effectiveness of cancer treatment, patient survival rates increase, and radiation oncologists are frequently faced with the problem of treatment of local recurrence or second tumors located within or close to previously treated sites. Initial dose influences different time intervals from tissue tolerance to re-irradiation as well as conditioning the recovery of radiation damage in the first treatment. It is possible to administer a higher dose in the re-irradiation if smaller doses were used at the first treatment and if the intervals between treatments were longer. Radiation myelopathy is a rare late toxicity effect in the modern era of 3-dimensional conformal conventionally fractionated RT. This devastating late effect has re-emerged as a direct result of SBRT practice, where high-dose radiation is delivered adjacent to the spinal cord to be spared. A comprehensive search was performed including relevant articles referring to “spinal cord”, “re-irradiation” and “myelopathy”. The biologically effective dose (BED) was calculated and the results are discussed considering radiobiological mechanisms.

Keywords: *Spinal cord, Radiobiology, Re-irradiation, Tolerance.*

Resumo

Avanços na biologia e na física permitiram uma maior precisão e exatidão em radioterapia (RT), de modo a maximizar o dano tumoral e minimizar as lesões nos tecidos normais adjacentes, que limitam a dose. A medula espinal é denominada como o órgão mais crítico em risco (OAR – *organ at risk*). A mielopatia por radiação é uma das complicações mais devastadoras da radioterapia clínica, resultando em morbidade grave e irreversível. A avaliação do impacto dos esquemas de dose e fracionamento, na tolerância tecidual, tem sido uma das principais áreas de pesquisa em oncologia da radiação. Como resultado de uma maior precisão e eficácia do tratamento oncológico, as taxas de sobrevivência do paciente aumentam e os oncologistas de radiação são, frequentemente, confrontados com o problema do tratamento de recidiva local ou de segundos tumores localizados dentro ou próximos de locais previamente tratados. A dose inicial influencia diferentes intervalos de tempo, desde a tolerância do tecido à re-irradiação, bem como, condiciona a recuperação do dano por radiação no primeiro tratamento. É possível administrar uma dose maior na re-irradiação se doses menores forem usadas no primeiro tratamento e se o intervalo de tempo entre o primeiro tratamento e a re-irradiação for mais longo. A mielopatia por radiação é um raro efeito de toxicidade tardia na era moderna da RT conformacional tridimensional convencional (3D- CRT). Este devastador efeito tardio ressurgiu como um resultado direto da prática de SBRT, onde a radiação de alta dose é administrada junto à medula espinal, que terá de ser poupada. Uma pesquisa abrangente foi realizada, incluindo artigos relevantes referentes a “medula espinal”, “re-irradiação” e “mielopatia”. A dose biologicamente efetiva (BED) foi calculada e os resultados são discutidos considerando os mecanismos radiobiológicos.

Palavras-chave: *Medula espinal, Radiobiologia, Re-irradiação, Tolerância.*

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Abbreviations

3D-CRT	Three-dimensional (3D) Conformal Radiation Therapy
AAPM	American Association of Physicists in Medicine
ACR	American College of Radiology
ALARA	As Low As Reasonably Achievable
ASTRO	American Society for Radiation Oncology
BED	Biologically Effective Dose
BSCB	Blood-Spinal Cord Barrier
Cdks	Cyclin Dependent kinase
CNS	Central Nervous System
CT	Computed Tomography
DNA	Deoxyribonucleic Acid
DSB	Double – Strand Breaks
EBRT	External Beam Radiation Therapy
ED_{50}	Effect Dose 50%
ERD	Extrapolated Response Dose
ETD	Extrapolated Tolerance Dose
EQD_2	Equivalent Dose of 2Gy
FSU	Functional Subunits
Gy	Gray
HR	Homologous Recombination
HVL	Half Value Layer
IGRT	Imaged Guided Radiation Therapy
IMRT	Intensity Modulated Radiation Therapy
IORT	Intraoperative Radiation Therapy
IPO-Porto	Instituto Português de Oncologia do Porto FG, EPE

kVp	kilo – voltage
LET	Linear Energy Transfer
LD	Lethal Dose
LQ	Linear–Quadratic model
MRI	Magnetic Resonance Imaging
NHEJ	Non – Homologous End Joining
NTCP	Normal Tissue Control Probability
OAR	Organ at Risk
OPCs	Oligodendrocyte Progenitor Cells
PNS	Peripheral Nervous System
RBE	Relative Biological Efficacy
RM	Radiation Myelopathy
RT	Radiation Therapy
RTOG	Radiation Therapy Oncology Group
SBRT	Stereotactic Body Radiation Therapy
Sec	second
SINS	Spinal Instability Neoplastic Score
SRS	Stereotactic Radiosurgery
SSB	Such as Single Break
Sv	Sievert
TCP	Tumor Control Probability
VCF	Vertebral Compression Fracture
VMAT	Volumetric Modulated Arc Therapy

Terms and Definitions

The glossary of terms and definitions has been adapted from: the book "Radiobiology for the Radiologist", the Council Directive 2013/59/EURATOM of 5 December 2013, the International Atomic Energy Agency (IAEA) TRS-398 and the online dictionary “The Free Dictionary” [1, 2, 3, 4]. This glossary has specific terms used in radiotherapy, radiobiology, radiological quantities and some clinical terms.

Absorbed dose – Measure of the energy imparted per unit mass by ionizing radiation to matter at a specific point. The SI unit of absorbed dose is joule per kilogram (J/kg). The denomination of this unit is gray (Gy). The previously used unit of absorbed dose, the rad, was defined to be an energy absorption of 100 erg/g. Thus, 1 Gy = 100 rad.

Absorption – Way in which the energy of a photon is taken up by matter, typically the electrons of an atom. Removal of x-rays from a beam.

Accelerated fractionation – The treatment schedule, in this case, exceeds the equivalent of 10Gy per week, in fractions of 2 Gy.

Acute hypoxia – Tumor region characterized by low oxygen concentration associated with changes in blood flow through the blood vessels may also be called *perfusion limited hypoxia*.

Adjuvant therapy – Type of treatment to combat cancer, used in addition to primary therapy. Usually, radiotherapy is used as an adjuvant for surgery or chemotherapy.

ALARA (as low as reasonably achievable) – Principle adopted to limit the dose of radiation to patients exposed to levels as low as reasonably possible, considering economic and social factors.

Angiogenesis – Denomination for the process of formation of new blood vessels.

Anterior – The ventral portion of a structure.

Apoptosis – A mode of rapid cell death after irradiation in which the cell nucleus displays characteristic densely staining globules and at least some of the DNA is subsequently broken down into internucleosomal units.

Bone marrow – The soft, organic, sponge like material in the cavities of bones; called also medulla ossium.

Cancer – Characterized by uncontrolled growth of the cells in the human body and the ability of these cells to migrate from the original site and spread to distant sites. If the spread is not controlled, cancer can result in death.

Cdks (cyclin-dependent kinases) – Proteins that complex with their cyclin regulatory subunits to phosphorylate proteins necessary for progression through the cell cycle.

Cell cycle checkpoint – Mechanism of control that acts to verify if each phase of the cell cycle was completed correctly before progression to the next phase.

Cells – Cells are the structural and functional units of living organisms. Each cell plays a specialized role in the body. Groups of cells are arranged together to form tissues. Tissues are organized to form organs in the body.

Central nervous system (CNS) – The portion of the nervous system consisting of the brain and spinal cord.

Dose – General term for the quantity of radiation.

Dose limit – Limit on dose that is applied for exposure of individuals to prevent the occurrence of deterministic effects and to limit the probability of stochastic effects.

Dose rate – Radiation dose delivered per unit time and measured, for example, in grays per hour.

ED_{50} (effect dose 50%) – Dose that produces the desired effect in 50 per cent of a population.

Effective dose – (E) is the sum of the weighted equivalent doses in all the tissues and organs of the body from internal and external exposure. The unit for equivalent dose is the sievert (Sv).

Equivalent dose – (HT) is the absorbed dose, in tissue or organ (T) weighted for the type and quality of radiation (R). The unit for equivalent dose is the sievert (Sv).

Fractionation – The daily dose of radiation based on the total dose divided into several daily treatments.

Free radical – A fragment of an atom or molecule that contains an unpaired electron, which, therefore, make it very reactive.

Function subunits (FSU's) – Many tissues can be thought of as consisting of discrete FSUs. These may be arranged in series as in the spinal cord, or in parallel as in the kidney.

Gray (Gy) – The special name for the SI unit of absorbed dose, kerma, and specifi c energy imparted equal to 1 J/kg. The previous unit of absorbed dose, rad, has been replaced by the gray. One gray equals 100 rad.

Grey matter – Part of the central nervous system consisting mainly of nerve cell bodies. The grey matter of the brain includes the outer layer (the cortex) and several centrally placed masses called nuclei. In the spinal cord, the grey matter occupies the central axis.

Homeostasis – The state of equilibrium, balance between opposing pressures, in the body with respect to various functions and to the chemical compositions of the fluids and tissues.

Hyperfractionation – The dose per fraction is less than 2Gy.

Hypofractionation – The dose per fraction is greater than 2Gy.

IMRT (Intensity Modulated Radiation Therapy) – Type of radiation treatment characterized with highly conformal dose distribution around the target using non-uniform beam intensities, which is possible using static or dynamic segments.

***In vivo* –** Occurring in an artificial environment.

***In vitro* –** Occurring within the living body of an organism.

Irradiation – Exposure to radiation, as in a nuclear reactor.

Late responses – Radiation-induced normal tissue damage that in humans is expressed months to years after exposure. The α/β ratio tends to be small, normally, > 5Gy.

Lethal dose (LD) – Dose of ionizing radiation enough to cause death. LD_{50} or MLD is the median lethal dose, what is the dose required to kill, within a specified period, half the individuals in a large group of organisms similarly exposed. For humans, $LD_{50/60}$ is about 4Gy.

Linear energy transfer (LET) – LET of charged particles in a medium is the quotient De/dl, where dE is the average energy locally imparted to the medium by a charged particle of specified energy in traversing a distance of dl [keV/µm].

Linear-quadratic model (LQ) – Used to describe the cell survival curve.

Metastasis – Occurs when cancerous cells invade surrounding tissues, enter the circulatory system and establish new malignancies in body tissues distant from the site of the original tumor.

Metastatic cancer – The stage of cancer is advanced in which cells from the primary site have spread, i.e., metastasized.

Misrepair (error prone repair) – Reconstitution with a loss of information (e.g., deletion caused by the loss of a fragment of the molecule or mutation or translocation).

Mitosis – Replication of a cell to form progeny cells with identical number (sets) of chromosomes.

Mitotic death – Cell death related with a post-irradiation mitosis.

Mitotic delay – As a result of treatment, delayed input into mitosis may occur due to accumulation of cells in the G2 phase.

Myelopathy – Any neurologic deficit related to the spinal cord. If its due to trauma, it is known as spinal cord injury. If it is inflammatory, it is known as myelitis. Disease that is vascular in nature is known as vascular myelopathy.

Oligonucleotide – DNA polymer composed of only a few nucleotides**.**

Oncologist – A physician who specializes in the study and treatment of neoplastic diseases, particularly in the treatment of cancer.

Oncology – Science dealing with the physical, chemical, and biological features of neoplasms, including causation, pathogenesis, and treatment.

Pathology – The branch of medicine treating of the essential nature of disease, especially of the changes in body tissues and organs that cause or are caused by disease. The study of diseased is realised both by gross and by microscopic examination of tissues removed during surgery and post-mortem.

Peripheral nervous system (PNS) – Part of the vertebrate nervous system constituting the nerves outside the central nervous system and includin g thecranial nerves, spinal nerves, and sympathetic an d parasympathetic nervous systems.

Posterior – Also called dorsal. Situated in the back. Opposite of the previous denomination.

Probability – A mathematical ratio of the number of times something will occur to the total number of possible occurrences.

Protocol – A detailed written set of instructions to guide the care of a patient or to assist the practitioner in the performance of a procedure developed specifically for tumors.

Radiation – Electromagnetic radiation consists of wave motion of electric and magnetic fields. The photons have neither mass nor charge and have an energy inversely proportional to the wavelength of the wave. The electromagnetic spectrum is divided into radio waves, infrared light, visible light, ultraviolet light, and x-rays, according to increasing photon energy and decreasing wavelength.

Radiation (ionizing) – Energy transferred in the form of particles or electromagnetic waves of a wavelength of 100 nanometres or less (a frequency of 3 x 1015 hertz or more) capable of producing ions directly or indirectly.

Radiation dose – The quantity of radiation what is absorbed for an irradiated object. It is expressed in gray (Gy), defined to be 1 J/kg.

Radiation quality – For high-energy photons produced by clinical accelerators the beam quality (Q) is specified by the tissue–phantom ratio, TPR20,10. This is the ratio of the absorbed doses at depths of 20cm and 10cm in a water phantom, measured with a constant source–chamber distance of 100cm and a field size of 10cm x 10cm at the plane of the chamber.

Radiation therapy – Use of ionizing radiation or any other type of radiation for the treatment of diseases. It is also called radiotherapy or actinotherapy.

Radiobiology – Study of the scientific principles, mechanisms, and effects of the interaction of ionizing radiation on living matter. Also called as radiation biology.

Radio-oncologist – Specialist physician with training in the use of radiotherapy, in order to reduce or cure patients with neoplasia.

Radiosensitivity – Susceptibility of cells, tissues, organs or organisms to the effects of radiation, such as x-ray or other radiation. Result of radiation effect.

Radiosensitizer – A chemical used to increase the radiosensitivity of cells to radiation. This substance mimics oxygen in fixing free radical damage.

Radiotherapy – Type of treatment to combat a neoplasia using ionizing radiation. This type of treatment has as main objective to give an optimal dose of radiation in the place of interest, to cause the smaller possible damages to the normal tissues. Also called radiation therapy.

Redistribution – Cells may exhibit different sensitivity depending on the phase of the cell cycle they are in. At the mitosis phase cells are more sensitive to DNA damage and late S phase are more resistant. With several dose fractions, there is progress in the cells through a new phase of the cell cycle.

Reoxygenation – Occurs only in tumor cells. Phenomenon where hypoxic cells become oxygenated after a dose of radiation.

Repair – Refers to the repair of the sublethal lesion. It occurs more efficiently in normal tissues, since tumor cells usually have more mitoses than the normal cells that generated them, uncontrolled cell cycle and

activation of the checkpoints for repair. Thus, by fractionating the treatment of the patient there is the possibility of repairing the normal tissues.

Repopulation – Growth capacity of tumor cells that escaped radio-induced death.

Save dose – is the maximum dose related to the body mass of a pharmacological agent that can be administered within 24 hours.

SBRT (Stereotactic Body Radiation Therapy) – Involves stereotactic localization techniques combined with delivery of multiple small photon fields in a few high–dose fractions, for extracranial treatments, leading to a highly conformal dose delivery with steep dose gradients. Stereotactic localization techniques may include the use of relocatable rigid frames, image–guidance techniques, and other positioning tools.

Spinal cord – Part of the central nervous system, along with the brain. It is characterized as being a thick cord of nervous tissue within the spinal canal. In humans it gives rise to 31 pairs of spinal nerves.

Stem cells – Non-specialized human cells that can produce all types of specialized cells in a lineage.

Syndrome – Combination of characteristics and symptoms that are indicative of a disease or disorder.

TCD_{50} – Radiation dose indicating that there is a 50% probability of tumor control.

Therapeutic index (therapeutic ratio) – Tumoral response for a permanent level of normal-tissue damage.

Tolerance – Maximum radiation dose prescribed by the therapist which is indicated as acceptable. It depends on several factors such as time between fractions, fractions indicated, field sizes and treatments previously performed.

Tumor – Growth of cells abnormally. Tumors can be benign or malignant (cancerous).

White matter – Constituted by myelinated nerve fibers. It belongs to the central nervous system (CNS).

Chapter 1

1. Introduction

Radiotherapy (RT) is a cancer treatment based on the use of ionizing radiation, where a major evolution occurred over the years. Advances in medical imaging, dose planning and treatment delivery allowed to maximize tumor damage and to minimize the damage in the adjacent normal tissues. Radiobiology has achieved a prominent role over the years, which has contributed to evolution of radiotherapy.

With improved delivery of radiotherapy treatments, survival rates have been increasing in many patients. This increase allowed the development of new tumors and local or regional recurrences, often within or near the previously irradiated site. When these situations occur, re-irradiation is a possibility that presents new challenges to radiation oncologists [5, 6].

One of the most important challenges posed by irradiation is the tolerance of organs at risk (OARs). Given that radiation has previously occurred, there are several factors to be considered so that complications in normal tissues do not overlap with the benefit that a new irradiation brings to the tumor. The spinal cord is considered an OAR because it is characterized by late complications that come from its irradiation, as is the case of radiogenic myelopathy. Since the spinal cord is the most dose-limiting organ in radiotherapy, it is important to understand what factors should be considered with re-irradiation. The tolerance of spinal cord irradiation depends on the irradiated volume, total dose, dose per fraction, time between treatments and the spinal cord region involved. In this way it is expected to control the occurrence of late complications associated to this organ that can give rise to devastating functional deficits [5, 6].

In this work, we first present a review of the actual knowledge about the spinal cord, such as its anatomy and physiology. A special attention is given to radiobiology and its role in a course of radiotherapy. With the advancement of medical technology and with a higher life expectancy of patients, local and regional recurrence often appears,

and it is necessary to prescribe re-treatments. Thus, this work intends to answer several questions, such as: what maximum dose can be given in the first treatment so that a re-treatment can be performed, what maximum dose can be prescribed in a re-treatment without causing severe late effects, what is the time interval between treatments, and which type of RT treatment best applies to the patient's condition.

Stereotactic body radiotherapy (SBRT) is an innovative RT treatment that aims to deliver with maximum precision the maximum possible radiation dose, in a small number of fractions (1 to 5 fractions) compared to conventional RT.

Chapter 2

2. Theoretical concepts

2.1 The role of radiation therapy

Radiation therapy (RT) is a common treatment for various cancers and uses ionizing radiation to damage the deoxyribonucleic acid (DNA) of malignant cells, which usually replicate at a faster rate than normal cells in the body. Ionizing radiation deposits energy in the material along its path, being absorbed by the same material through various interactions. When radiation interactions occur, it breaks the molecular bonds of the DNA of the cells thus altering its structure. Through this mechanism, radiotherapy can prevent the replication of abnormal cells causing cellular dead [7, 8]. The different types of radiotherapy are shown in (Table 1).

Table 1 Types of radiotherapy according to the type of target. Adapted from [5].

Types of radiotherapy		
Preventive radiotherapy	Curative radiotherapy	Palliative radiotherapy
Prevention of possible metastases or recurrences through the application of radiotherapy.	Tissue–tumor ratio is such that curative doses of radiation can be used without unduly harming normal tissue.	Radiation therapy can be administered to relieve pain in cancer patients.

Radiotherapy may be used as a single or neoadjuvant treatment (treatment given prior to any other treatment) or adjuvant (treatment given after any other type of treatment such as surgery and chemotherapy) [7].

RT can be administered in the following ways (Table 2): internal radiotherapy (brachytherapy, intraoperative radiotherapy (IORT)) and external radiotherapy (stereotactic body radiotherapy (SBRT), three-dimensional conformal radiotherapy (3D-CRT), radiotherapy modulated by intensity (IMRT), image-guided radiotherapy (IGRT) and radiosurgery).

Table 2 Description of how radiotherapy can be administered to the patient [7].

Type of treatment	Description
Brachytherapy	Uses radioactive sources (temporary or permanent) placed near the target volume. These sources can be inserted directly into the tumor or placed through applicators previously inserted into a body cavity.
Intraoperative radiotherapy (IORT)	Administered under intraoperative conditions, usually by electron beams or low energy x-rays. It is used after resection of the primary tumor and external radiation therapy is usually necessary.
External radiotherapy	It involves the use of a linear accelerator to administer, outside the patient's body, radiation beams focused on the target volume to be treated.
Stereotactic body radiotherapy (SBRT)	Administered through multiple beams that are focused on a three-dimensional target. For tumors of the central nervous system (CNS) a thermoplastic mask is used, whereas for extracranial sites a body frame may or may not be used.
Three-dimensional conformal radiotherapy (3D-CRT)	Technique used where dose volume is made to fit the target using 3D anatomical data acquired from computed tomography (CT) or magnetic resonance imaging (MRI) imaging modalities. The goal is to apply the maximum dose to the target and save neighbouring structures as much as possible with the help of advanced computer hardware and software.
Radiotherapy modulated by intensity (IMRT)	It provides a highly conformal dose distribution around the target using non-uniform beam intensities, which is possible using static or dynamic segments. The isodose distribution can then be closely monitored by the target, modulating the intensity.
Image-guided radiotherapy (IGRT)	It uses various radiological and functional imaging techniques to perform high-precision radiotherapy. The main goals are to reduce the configuration and internal margins, and account for changes in target volume during radiation therapy, such as decreased tumor volume or weight loss (adaptive radiotherapy).
Radiosurgery	Can be performed by all kinds of advanced radiotherapy techniques, including IMRT, IGRT, synchronized respiratory radiotherapy, tumor-tracking radiotherapy and SRS/radiotherapy.

The main objective of radiotherapy is to administer the prescription dose in the target volume, saving as much adjacent normal tissues as possible [7].

2.2 Radiobiology: essential concepts

In radiation oncology, radiobiology is defined as the science that investigates the interactions between ionizing radiation and living systems, as well as the consequences of these interactions [7].

2.2.1 The role of radiobiology in the evolution of radiotherapy

Radiobiology has allowed creation of new ideas and identification of potentially exploitable mechanisms in radiotherapy. According to experimental and theoretical studies in radiobiology, it was possible to verify that this area contributed through three different levels for the development of radiotherapy [9]:

- *Conceptual basis for radiotherapy*: identifying the mechanisms and processes underlying the response of tumors and normal tissues to irradiation, which lead to the explanation of observed phenomena. The knowledge about the 5 Rs of radiotherapy is an example of the knowledge acquired through the conceptual basis;
- *Treatment strategies*, allowing the development of new approaches in radiotherapy. Examples are the discovery of hypoxic cell sensitizers, high linear energy transfer (LET) radiotherapy and hyperfractionation;
- *Protocols*, providing a more diverse range of treatment schemes in clinical radiotherapy.

These three levels used by radiobiology are a starting point that provide insight into new options. Many treatment strategies produced through radiological investigation do not produce demonstrable clinical benefits. Creating protocols through experiments is a slow process. The ability of laboratory science to guide a radiation oncologist in the choice of specific protocols is limited by the inadequacy of theoretical and experimental models to clinical practice [9].

2.2.1.1 Radiobiological mechanisms

In the exposure of living tissues to ionizing radiation absorption of photon energy by cells occurs. The radiation through the biological material will trigger a series of events, interact with the atoms and molecules of the medium, with the consequent transfer of energy.

There is strong evidence that DNA is the target of the biological effects of ionizing radiation, namely cell death, loss of clonogenic capacity, genetic mutations and

chromosomal aberrations, with consequent somatic, hereditary, teratogenic and carcinogenic effects.

Ionizing radiation causes damage to cells or tissues by depositing energy in a sequence of events. Different types of radiation have distinct abilities to cause biological damage. All these lesions are caused at the cellular or molecular level. The biological effects subsequently manifested are due to the impact of these lesions on millions of cells in an organ or tissue. Considering that the nature of the damage is molecular or cellular, it is therefore essential to understand the mechanisms involved.

Ionizing radiation, used in radiotherapy, causes a cascade of events, which begins immediately after its emission. The initial ionization (*physical phase*) is followed by immediate damage of vital macromolecules at the cellular level – **direct effect**, or indirectly through interaction with water molecules, resulting in free radicals of oxygen, highly reactive at the molecular level (*physical–chemical phase*) – **indirect effect**.

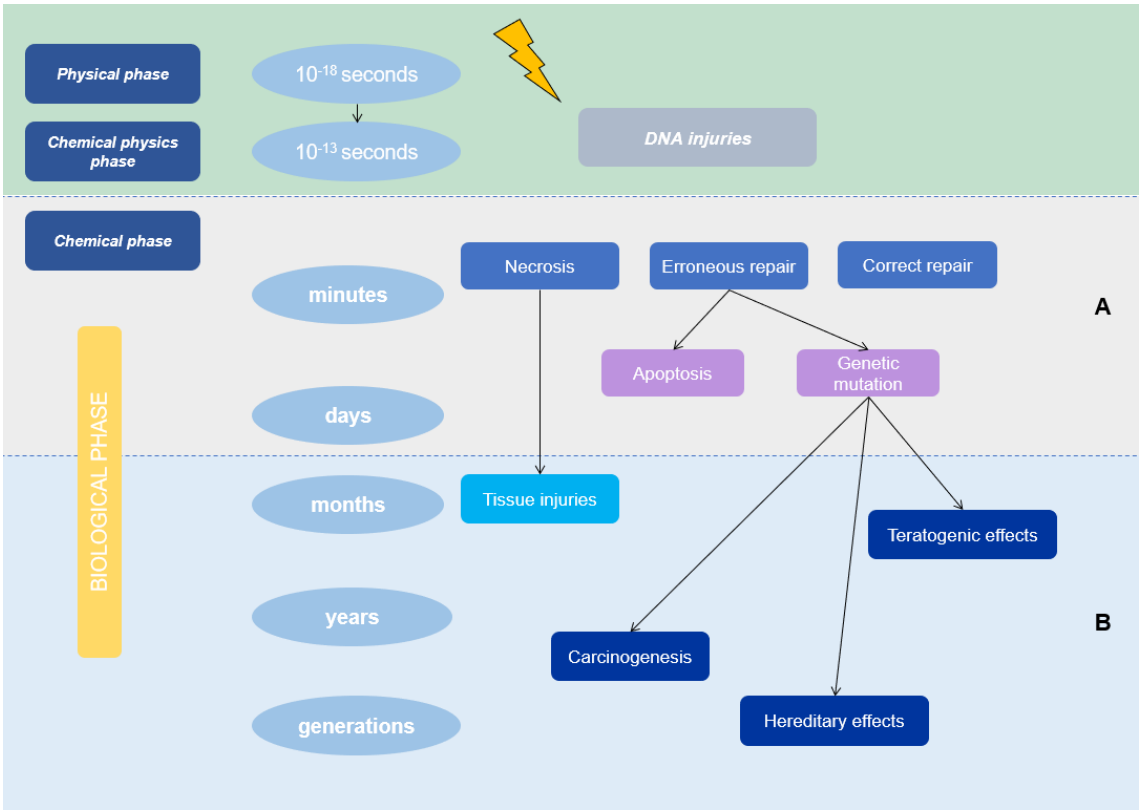


Figure 1 Time scale of the effects of ionizing radiation: biological changes manifest after a period of latency that can go from minutes to weeks to years after exposure (A - early effects; B - late effects).

The energy deposited initially by ionizing radiation is used in the formation of free radicals. Since the cells are mainly composed of water, most of the lesions caused by x-rays to biological molecules - about 2/3, are mediated through free radicals, namely hydroxyl radicals. The free radicals resulting from these reactions initiate complex

chemical reactions, which can lead to the destruction or inactivation of vital molecules in the cell. Chemical phase follows in which sub-lethal damage repair can occur. Then, during the biological phase, the manifestation of unrepaired lesions in the DNA occurs.

These 3 different phases described previously differ in time scale (Figure 1) [9]:

1) *Physical phase* (about 10^{-18}sec) – phase where photon energy deposition occurs to the orbital electrons, exciting or ionizing them;
2) *Physical–chemical phase* (about 10^{-13}sec) – the breakdown of chemical bonds caused by excited or ionized atoms occurs. These atoms can cause damage to important molecules, such as DNA, in a direct way or through the formation of free radicals;
3) *Chemical phase* (seconds to years) – constituted by the reaction of the cells to the damages caused by the radiation. Damage to DNA can be repaired by specific enzymes, but some are irreparable leading to cell death. Cell death is not immediate occurring in most cell division after irradiation (and may occur up to 5 or 6 cycles after).

The main function of radiobiology is to observe the phenomena that occur with irradiation of the tumor and normal tissues, suggesting improvements to the existing therapeutic options. The tumor response to irradiation is called regression and may be followed by recurrence. If tumor recurrence does not occur during the patient's life, local tumor control may be considered to exist [9].

Thus, when a cell absorbs radiation 4 possibilities can occur:

- Absorption of the radiation may have no adverse effects, or if injury occurs, it may be repaired without any trace of exposure occurring; if not repaired, the apoptosis pathway may be activated;
- Cell can suffer lethal, irreparable and irreversible damage, leading to cell death;
- Cell may lose its clonogenic capacity;
- Gene mutations may occur, with distinct consequences depending on the type of cell where they occur.

The most frequent cause of radiation-induced cell death is the inability to correct double lesions in the DNA strand and manifests itself when the cell attempts subsequent cell division. In this way, more proliferative cells manifest damage or die much earlier than cells with longer proliferation times. Thus, biological changes in cells and tissues due to ionizing radiation occur only after a latency period, which may range from minutes to

weeks or even years (as a function of dose, dose rate, cell kinetics, control of cycle regulating genes cell phone, etc.).

Genetic and biochemical constitution is a determining factor in the molecular response to radiation, and several molecules are already identified through which cells detect radio-induced lesions.

Recognition of these lesions activates signal transduction pathways suitable for cellular response to injury. This process is influenced by internal cell signalling processes as well as external factors such as hypoxia, cytokines, intercellular contact and extracellular matrix. The result of these interactions may promote cell survival or death, cell cycle arrest or blockage, and DNA repair or genetic instability, depending on how the cells respond to radio-induced lesions.

Thus, while initial energy deposition and subsequent events occur in 10^{-18}–10^{-13} sec, the chain of biological effects that begins by inducing programmed cell death or repairing sublethal and potentially lethal damage, leading to tissue repair and remodelling can take minutes, hours, days, months or years to express these effects.

There are also several factors that affect the cell response to radiation: physical factors (dose, dose rate, fractionation, LET - linear energy transfer - and RBE - relative biological efficacy); chemical factors (radiosensitizers, radioprotectants, O_2 tension) and biological factors (proliferative state, cell cycle phase, physiological or metabolic state, genetic constitution of the cell) - reviewed in [10].

2.2.1.2 Cell cycle control

The concept of cell cycle is essential for the knowledge of all cellular radiobiology processes. It is a necessary process that involves great fidelity and of extreme importance for the propagation of organisms (Figure 2).

The cell cycle consists of a succession of events that lead to duplication of genetic material and other cellular components and eventually cell division (Table 3).

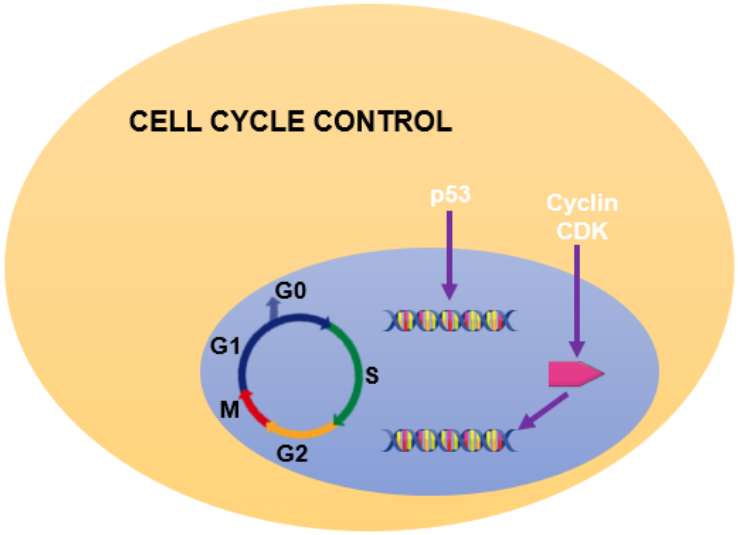
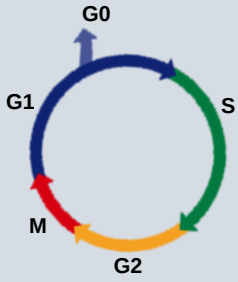


Figure 2 Role of cyclins and cyclin-dependent kinases (CDKs) in cell cycle regulation: The cell cycle is divided into G1, S (DNA synthesis), G2 and M (mitosis) phases. The transition between phases is controlled by cyclins and CDKs.

Table 3 Phases of the cell cycle: representation and description.



Cell cycle	Description
G1 phase (gap)	The synthesis of enzymes involved in DNA replication occurs;
S phase	The cell generates an integral copy of the genetic material, proceeding to a second preparatory phase of cell division;
G2 phase	Occurs before entering mitosis.
Mitosis (M)	The DNA replicate is condensed into compact chromosomes, segregated into the daughter cells so that each one receives a complete copy of the genetic material.
G0 phase	Period in which the quiescent cell maintains its metabolic activity but does not grow unless it receives extracellular signals.

For cell cycle progression to occur, a DNA check system is required to ensure complete duplication of the genome in an orderly and highly faithful manner. Changes in normal cell cycle control lead to genetic instability, a major factor in carcinogenesis. This is why the existence of checkpoints for the integrity and state of the replication of the genetic material that allows the stop of the cell cycle progression in case of damage or mutation of the DNA, so that it is evaluated and repaired, ensures the complexity and irreversibility of the cell cycle, as well as the processes of activation, expression and degradation of the proteins involved. On the other hand, physiological cell death in self-regulating tissues, such as the skin, intestine and bone marrow, is necessary to give rise to the cells that are constantly formed: it is programmed cell death also called apoptosis. In this

process, where several regulatory genes and a family of proteases, the caspases, are involved, the cells are fragmented into membrane-bound corpuscles, with consequent phagocytosis by neighbouring cells, with no inflammatory response.

Failure to induce apoptosis contributes to carcinogenesis by allowing the occurrence of genetic instability and the deregulation of the activity of the genes involved in cell cycle control and their checkpoints. During the regulation processes, the cell activates and inactivates the proteins by addition by the kinases or removal by the phosphatases of the phosphate groups, respectively. For faster and more effective kinases and phosphatases can physically bind to the protein they modify, with formation of multiprotein complexes [11].

The p53 gene, known as the "guardian of the genome", is a tumor suppressor gene that encodes a nuclear phosphoprotein that, because of its physiological functions, ensures cellular genetic integrity: cell cycle regulation, apoptosis control and DNA repair (Figure 3).

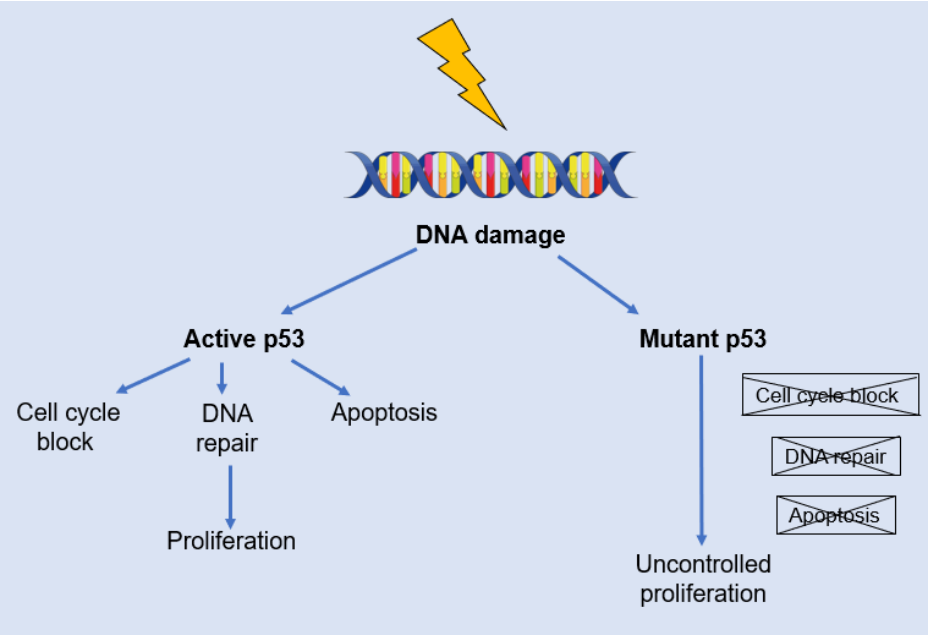


Figure 3 p53 Injury recognition process: under normal conditions the protein expressed by the p53 gene is responsible for the temporary stopping of the G1 cell cycle for DNA repair or, if not possible, programmed cell death (apoptosis). If the gene is mutated, the functions of p53 are not activated and the result will be uncontrolled cell proliferation.

When DNA damage occurs, the p53 protein is responsible for the temporary stopping of the cell cycle in G1 for repair or, when this is not possible, the process of cell apoptosis begins. Thus, if p53 mutation occurs, the functions of the protein may not be activated, and the consequence will be uncontrolled cell proliferation. Mutations of this gene are described in more than 50% of human tumors, conferring a proliferative advantage on

cells carrying the mutation. Apoptosis is one of the best studied processes in cell death by ionizing radiation and depends on p53. Cells with mutations in this gene do not undergo apoptosis after treatment with ionizing radiation, unlike in cells where the p53 gene is active [12].

2.2.1.3 Proliferation and differentiation

The concept of cell proliferation and its influence on response to treatment is crucial in RT. Cell proliferation in normal tissues, unlike in tumors, is very well organized through homeostatic control: there is a balance between cell production and the loss of more differentiated cells. Radiation is a unique cytotoxic agent because lesions caused to DNA can remain inactive for hours, days, weeks or months. This occurs because in the clear majority of normal and tumor tissues, cell death only occurs when the cell attempts the next division, which depends on the proliferative characteristics of the tissue. In cells of the intestinal mucosa, in which cell cycle time (Tc) is short (12-24h), cell death occurs after a few hours: in the skin (Tc = 4 days), may take a week; in the kidney (Tc indeterminate) can take months [13].

As at the cellular level there are preferential targets for the action of radiation, also at the tissue level there are cells or groups of cells more sensitive or whose death depends on the changes observed in the body after irradiation. The different organs or tissues of the living organism are composed of several cell types and the response of an organ or tissue to the radiation depends on the intrinsic sensitivity of the different cellular populations in that organ or tissue and the proliferative characteristics of each population.

The Law of Rubin and Casarett differentiates tissue sensitivity – being that this is essentially a function of the type of cell that constitutes the tissue – in five distinct groups. In the first group are the most sensitive cells, as they are those that are more metabolically active, divide more quickly and more undifferentiated. In the second group are the differentiated, intermittent cells, the result of the cell division of the cells of the previous group, which are still very active and relatively undifferentiated mitotically. In the intermediate group, which includes connective tissue cells, we find vascular endothelial cells and fibroblasts. In the following groups cell differentiation increases and proliferation decreases until the last classification, which includes fixed postmitotic cells, where we find examples of the most resistant cells [14].

2.2.1.4 Tolerance of normal tissues to radiation

Cellular organization in proliferative and functional compartments has important consequences for tissue response to radiation. In this way, the tissues can be divided into 2 categories.

In the first category - **hierarchical cell organization** - tissues that have a clear separation between the proliferative compartment (including the population of stem cells, capable of unlimited self-renewal; the amplification compartment - cells that proliferate rapidly but only over a limited number of cell divisions), and the differentiated cell compartment, responsible for organ / tissue functions. As examples we have the most proliferative tissues such as the hematopoietic system, the cells of the basal layer of the epidermis and the lining of the gastro-intestinal system and the spinal cord.

The begin of the acute reactions of these tissues to the radiation is correlated with the life span of the differentiated functional cells, and the intensity of these reactions reflects the ratio between the rate of destruction of the stem cells and the rate of regeneration of the surviving clonogenic cells.

In the second category - **flexible cell organization** - tissues are included in which there is no clear separation between the two compartments but in which some differentiated cells also exhibit self-renewal capacity. In this type of organ/ tissue with a low proliferation rate (kidney, lung), the relationship between cell death and tissue response is less evident because organ damage can occur due to changes in vascular, connective or parenchymal tissue.

Late effects are not only restricted to these slow cell renovation tissues. For example, in epithelial tissue, late lesions - fibrosis, atrophy and telangiectasia - may occur in addition to early reactions. Thus, different types of lesions may occur sequentially in an organ or tissue, resulting from distinct mechanisms and cellular interactions.

The difference between acute and late effects can be explained by their progression: while the acute effects are quickly repaired by the high proliferation of stem cells and can be completely reversible, the late effects can be attenuated but never completely repaired as they result from the association of vascular lesions with loss of parenchymal cells.

This distinction has relevant biological consequences: as acute reactions occur during conventional RT treatment, it is possible to make the necessary changes to allow the survival of stem cells that will repopulate and ensure cell proliferation. Late effects, which

occur months or years after RT, are much more sensitive to changes in fractionation than early effects.

Tolerance of normal tissues may also be influenced by treatment-related variables (total dose and dose per fraction, dose rate, total time of treatment, energy and volume irradiated, use of concomitant chemotherapy), patient (age, comorbidity associated with diabetes, vascular disease), or with the organ in question (development of radiation toxicity, variation in intrinsic radiosensitivity of the organ) - reviewed in [15].

2.2.1.5 Rs of radiobiology

The complexity of the response to RT increases with the characteristics of the surrounding normal tissues: while cells and tissues may respond differently to the same dose of radiation, the response of a tissue is strongly determined by the rate of cell proliferation and tissue repopulation capacity in addition to the molecular and cellular factors that determine intrinsic cellular radiosensitivity.

Tumor responses show greater variability than normal tissues and these biological differences are explored in dose fractionation in radiotherapy, where the protocols are derived empirically but exploit differences in biological response between normal and tumor tissues at the same dose of radiation. The biological factors that influence the response of normal and tumor triglycerides to RT were summarized by Withers (1975) as the radiotherapy Rs [16].

Radiotherapy given in a single, high dose fraction is ineffective for tumor control and has serious side effects. To reduce these effects, radiotherapy was given in small fractions daily and at low doses and this type of treatment was referred to as fractional radiotherapy [7].

Fractional radiotherapy is based on five main features known as the "Rs of radiobiology". These are described then in order of occurrence, i.e., the first biological mechanism observed is the repair of sub-lethal damage, followed by redistribution of cells in the cell cycle, reoxygenation, and finally repopulation [7].

The classical fractionation principles, i.e., the Rs of radiobiology, explain the effects of high doses of ionizing radiation on tumors and adjacent normal tissues. The outcome of standard clinical radiation treatment is determined by the Rs of radiobiology.

2.2.1.5.1 Repair of sub-lethal damage

In the literature, this mechanism can be called in several ways: "repair", "repair of DNA damage" or "repair of sub-lethal damage".

Repair of sublethal DNA damage: normal cell are more effective than tumor cells in this process as observed from cell recovery in the 2 hour period after exposure to ionizing radiation. Radiation randomly interacts with molecules in the cell, but DNA is the main target molecule for the biological effects of radiation, including cell killing, carcinogenesis and mutation. In radiotherapy radiation damage is primarily manifested by the loss of reproductive capacity. Radiation causes a wide range of lesions in DNA such as single (SSB) and double-strand breaks (DSB) in the sugar-phosphate backbone of the DNA molecule. SSB can be readily repaired using the undamaged chromatid as a template. The most deleterious lesion induced by ionizing radiation is DSB, a break in both strands of 10 base pairs or less. There are several mechanisms to repair DSBs, which indicate the importance and difficulty of repairing this type of DNA injury. The most important are non-homologous end joining (NHEJ) and homologous recombination (HR). Homologous recombination provides greater repair accuracy than NHEJ, the major pathway to repair DSB throughout all the phases of the cell cycle [17].

Radiotherapy cause lethal damage to tumor cells and sublethal damage to normal tissue cells. Sub-lethal damage can be repaired if enough time is given between exposures to radiation. If the cell is exposed to radiation before the repair occurs, the damage can become lethal, i.e., the sum of the damages caused leads to the repair not being viable and cell death occurs (Table 4) [7].

Normal tissue cells that have a late response to radiation can repair faster than tumor cells, if there is an ideal range between fractions of 6-12h [7].

Different types of cells have distinct abilities to correct radiation-induced damage, and some cells having been verified to be faster compared to others to repair sub-lethal damage [9].

Table 4 Characteristics of different types of cell death. Adapted from [9].

	Morphological changes			
Types of cell death	Nucleus	Cell membrane	Cytoplasm	Biochemical features
Apoptosis	Condensation of chromatin, nuclear fragmentation; DNA laddering	Blebbing	Fragmentation	Caspase-dependent
Autophagy	Partial chromatin condensation;	Blebbing	Autophagic vesicles	Caspase-independent
Necrosis	Degradation of nuclear DNA	Swelling and rupture	Organelle degeneration and mitochondrial dilatation	-
Senescence	Distinct heterochromatic structure	-	increased granularity and flattening	SA-β-gal activity
Mitotic catastrophe	nuclear fragmentation and dicentric chromosomes.	-	-	Caspase-independent (at early stage)

It is relevant to distinguish two processes that are commonly accepted as the same: repair and recovery. While the first refers to the method in which the cell corrects a radiation-induced error, recovery is understood as the ability of a tissue, not a cell, to increase its cellular survival or decrease the damage caused if it has sufficient time for this process to occur, such as the recovery of an erythema.

Repair is due to the correction of sub-lethal damage and can be measured, for example by a sequence of irradiances separated by variable time interval. On the other hand, recovery may involve the recruitment of cells that are in a non-division phase, G0, to enter the cell cycle and thus compensate for cell death [9].

2.2.1.5.2 Redistribution of cells in the cell cycle

Cell cycle consists of four distinct and consecutive phases (Figure 4) [9]:

- Phase G1 and G2, which are periods of apparent inactivity (gap), where the G1 phase occurs before S phase and the G2 phase between synthesis and mitosis;
- Phase M corresponds to the phase of mitosis;
- Phase S, which is the period where the synthesis of genetic material occurs to proceed to the division.

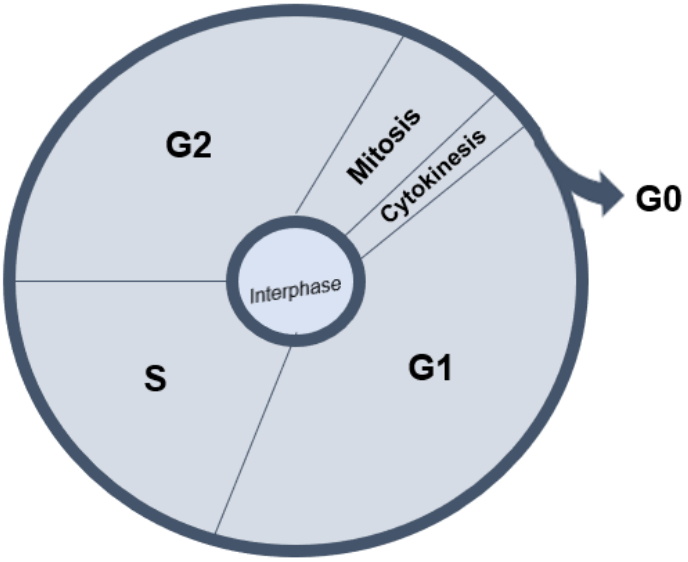


Figure 4 Representation of the cell cycle. Adapted from [18].

Redistribution: when radiotherapy is given to a heterogenous cell population, cells may be in different phases of the cell cycle. Cells in S phase are more radioresistant and cells in late G2 and M phases are more sensitive. A small dose of radiation will destroy the more sensitive cells, and a resistant cell population that is now synchronized survives. As fractionated radiotherapy treatment continues, the resistant surviving cells will continue throughout the cell cycle and when a new dose is delivered some of these cells have moved from a resistant to a more sensitive stage and will then be killed more easily (Figure 5) [19].

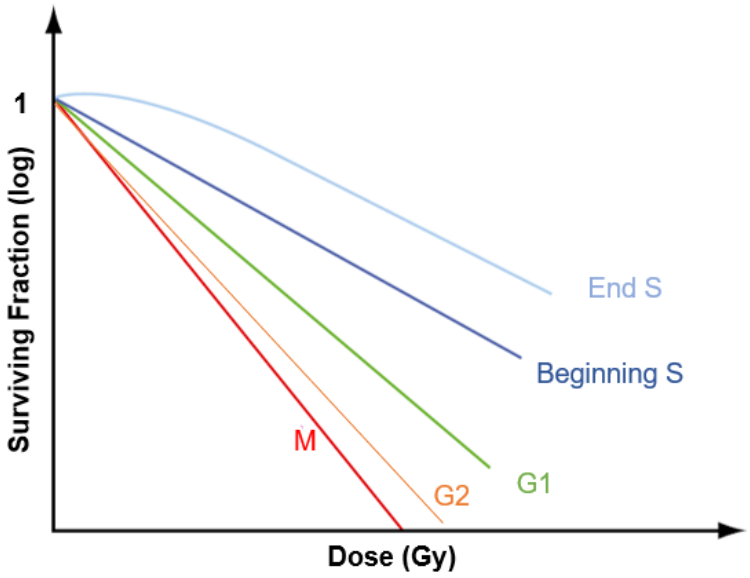


Figure 5 Cell cycle and survival curve phases. Adapted from [7].

2.2.1.5.3 Reoxygenation

As tumor volume increases through tumor cell proliferation, vascularization does not accompany this tumor growth, becoming insufficient to meet its requirements and hypoxic regions begin to appear in tumor tissue. Hypoxic cells are 2-3 times more resistant to radiation, and well-oxygenated cells are eliminated during treatment of fractionated radiotherapy. Since oxygen supply is constant, hypoxic cells gradually gain vascularity and oxygenation, and the output radiosensitivity increases [7].

Reoxigenation: The level of oxygenation in a tumor is a major determinant of the effectiveness of radiotherapy. Tumor cell microenvironment presents areas with decreased pH, lack of nutrients and hypoxia. Oxygen concentration (pO2) varies between 10- and 80-mm Hg in normal tissues but in tumors these values can be lower than 5 mm Hg in some areas. This anomaly is due to the development of abnormal vasculature during tumor angiogenesis. A significant proportion of tumor cells is hypoxic, showing great heterogeneity that is not correlated with standard prognostic factors such as size, stage and histological type [20].

Hypoxia may have a crucial role in treatment outcome and may also influence metastatic capacity of tumor cells resulting from genetic changes such as those involving blood oxygen transport or inducing vascularization. As a result of prolonged exposure to hypoxia, cells can acquire genetic resistance to apoptosis suggesting that hypoxia can favour tumor progression through clonal selection of cells with more aggressive phenotypes [21].

After an initial dose of radiotherapy, the more sensitive oxygenated cells are killed; during reoxygenation, surviving tumor hypoxic cells can increase their oxygen supply thus increasing their sensitivity to radiation. Biological efficacy of ionizing radiation relies on oxygen interacting with cells and making DNA lesions permanent [22].

Prolonged exposure to hypoxia can induce tumor death by apoptosis, as cells with mutations in the p53 gene acquire genetic resistance to hypoxia-mediated apoptosis, suggesting that hypoxia may favour tumor progression by selecting cells with mutations in p53 [19]. Other studies suggest that cells in hypoxia may develop genomic instability or that these cells may reduce the functionality of proteins involved in DNA repair [23].

After a first dose in the treatment of RT, the more sensitive oxygenated cells are eliminated; the surviving tumor cells are in hypoxia but later, during treatment, their O_2 supply can improve, increasing the sensitivity to radiation. The biological efficacy of

ionizing radiation depends on the presence of oxygen, which reacts with the cells making the DNA lesions permanent [24].

The division of the dose into several fractions spares the normal tissue due to the occurrence of repair of the sublethal damages and the cellular repopulation that occurs between the fractions. Simultaneously, fractionation allows for greater damage to the tumor caused by reoxygenation of hypoxic cells and the redistribution of cells to more radiosensitive phases of the cell cycle.

To oxygenate a tumor in the hypoxic state [7]:

- If the haemoglobin value is low, a blood transfusion can be given to the patient;
- High pressure oxygen may be applied during radiotherapy;
- The patient may be prevented from using hypoxic materials, such as cigarettes, during radiation therapy;
- Hypoxic radiosensitizers may be used, such as metronidazole.

2.2.1.5.4 Repopulation

Repopulation: Some time after irradiation an increase in cell division is seen in normal and malignant cells. Repopulation occurs at different rates depending on the tissues and represents cell proliferation that aims at compensating the cell population that was killed.

This homeostatic response to cell loss occurs in situations other than irradiation and is related to specific cell-cycle time: as a result of radiotherapy cell death occurs after irradiated cells attempt mitosis and thus highly proliferative tissues (and tumors) show damage much faster than slowly proliferative tissues.

Normal and tumor cells continue to proliferate when exposed to radiation. This proliferation is a physiological response of tissues to a decrease in cell numbers. The proliferation of the cells leads to two main consequences, such as the increase in the number of tumor cells to be destroyed, which is against the stipulated treatment, and the increase in the number of normal cells after irradiation, which is in favour of the treatment [7].

Repopulation allows tumor cells to resist the lethal effects of radiation therapy.

2.2.1.5.5 Radiosensitivity

The fifth "R", radiosensitivity is a concept that can be affected by the cell microenvironmental conditions. *Bergonie and Tribendau*, in 1907, defined that radiosensitivity was directly proportional to mitosis and inversely proportional to cell differentiation [7].

To summarize, considering t as the time between fractions and T the total treatment time (Table 5) [7]:

Table 5 Influence of time t and T according to the "R". Adapted from: [7].

Rs	Description
Repair	T should be minimum for normal tissues
Redistribution	t should be minimum
Reoxygenation	T should be minimum
Repopulation	T should be minimum for the tumor

Dividing total dose in several fractions saves normal tissue due to sublethal damage repair and cell repopulation occurring between treatment fractions. At the same time fractionation allows greater damage to the tumor as a result of reoxygenation of hypoxic cells as well as redistribution of cells into more radiosensitive stages of the cell cycle. Therefore, DNA repair and cell repopulation mechanisms induce normal tissues to become more resistant to a following dose of radiation; the other Rs, namely redistribution and reoxygenation have the opposite effect increasing radiosensitivity of tumor cells. The Rs represent the factors that modify tissue response to fractionated radiotherapy - overall radiosensitivity of a specific tumor depends upon a 5^{th} R: Radiosensitivity [25], that translates the outcome of all the other Rs and represents individual sensitivity to radiation. These five fundamental factors represent the biological basis of radiotherapy.

2.2.2 Cell survival curves

The cell survival curve describes the ratio between the fraction of surviving cells, i.e., the fraction of irradiated cells that maintains their reproductive integrity, and the dose absorbed. Cell survival is plotted with the dose on the x-axis and the fraction of surviving cells on the y-axis [24].

The number of cells inserted into cell lines and cultures can increase in two ways: arithmetically and exponentially (Figure 6). The number of cells increases linearly with each generation in an arithmetic increase. On the other hand, in the exponential increase, the number of cells doubles with each generation and thus the exponential growth is faster when compared with the arithmetic growth [7].

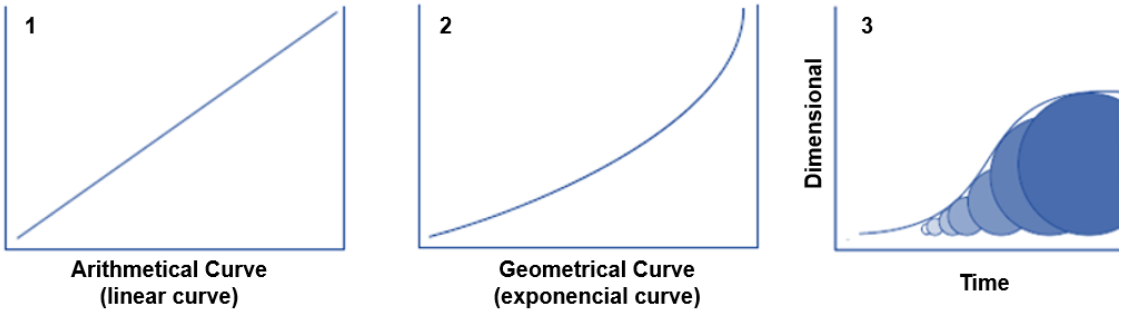


Figure 6 Curves of cellular survival: 1) arithmetic, 2) geometric and 3) Exponential increase in cell number. Adapted from [7].

The type of radiation used influences the way the cell survival curve takes. The ionizing radiations that have high linear energy transfer (LET) (densely ionizing radiations) result in a curve similar to an exponential function. On the other hand, ionizing radiations that have low LET (sparsely ionizing radiations) present curves with an initial inclination and become almost straight when the doses are higher [26].

The factors that make cells less radiosensitive are [26]:

- Removal of oxygen, making the cells hypoxic;
- Addition of chemical components as free radical catalysts;
- Use of low doses or fractional irradiation;
- Irradiation of cells in the late S phase of the cell cycle.

Cell survival curves are an example of dose-response curves and are widely used in the field of radiobiology when developing the Linear Quadratic model [9].

According to the linear quadratic model (LQ), the fraction of surviving cells can be calculated (Eq.1) [26]:

$$S(D) = e^{-\alpha D - \beta D^2} \ (Eq. 1)$$

Where:

- S represents the fraction of surviving cells at a dose (D);
- α is a constant that describes the initial inclination of the survival curve;
- β is a small constant that describes the quadratic component of cell death.

From the analysis of the cell survival curve it is possible to extract information on both the number of cells killed by the ionizing radiation and the radiosensitivity of the cell. In this way, it is important to define the parameters α and β. These are both radiosensitivity parameters, since α is the parameter that measures the amount of lethal damage and β is the parameter that measures the amount of sublethal damage [7, 9].

2.2.3 Dose-response relationship

The purpose of radiotherapy is to provide enough radiation to the tumor to destroy it, considering the protection of the surrounding normal tissues. These tissues should be protected from a dose that can lead to serious complications, i.e., morbidity [9].

When there is a change in treatment strategy it is necessary to consider both the effects on tumor response and damage to normal tissues. By analysing these two parameters it is possible to evaluate the existing options. If on the one hand the benefits are related to the tumor response, on the other the damages are evident in the normal tissues. There are also other factors that weigh on this type of decision, namely little quantifiable aspects such as new forms of toxicity or risks to the patient, among others. The role of radiobiology is to ensure an approach to the quantifiable biological aspects inherent in a change of treatment [9].

Radiobiology applied to clinical radiotherapy has as main interest the relation between a certain absorbed dose of radiation and the biological response. As the radiation dose increases, the radiation effects can increase in degree and/or incidence. In most cases, the interest is to know the relationship between dose and incidence. The dose-response curves take a sigmoid (S) form, with the incidence tending to zero as the dose tends to zero and the incidence tends to 100% at very large doses. This applies to tumor control as to normal tissue complications [21].

The tumor control probability (TCP) is plotted as a function of the total dose and the incidence of normal tissue complications is also plotted as a dose function. The exposed figure 7 represents a favorable situation because it shows that the tumor is more radiosensitive compared to normal tissue [21].

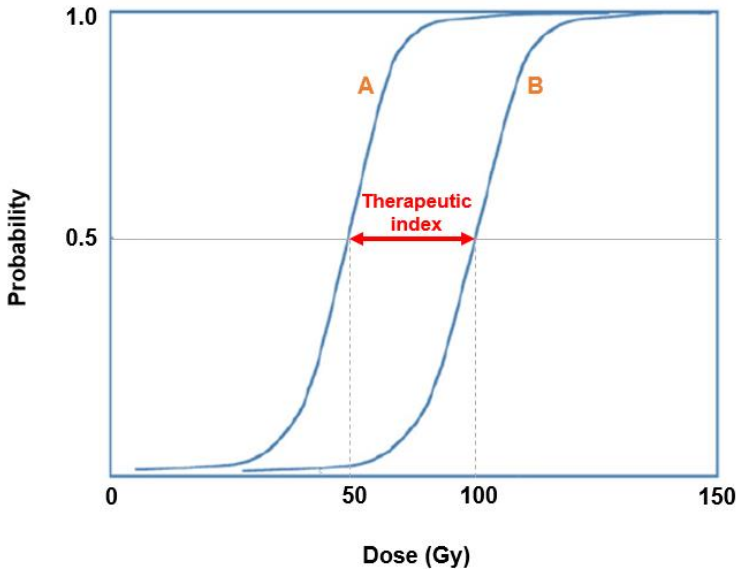


Figure 7 Principle of therapeutic index. Curve A: Probability of tumor control (TCP); Curve B: Probability of complications (NTCP). Adapted from: [21, 26].

The optimal choice of the radiation dose delivery strategy in a tumor occurs when TCP is maximized and NTCP is minimized. For a good radiotherapy treatment TCP ≥ 0.5 and NTCP ≤ 0.05. Observing figure 7, we can see that as the dose of radiation increases, the tumor response increases and so does tumor tissue damage and consequent complications [26].

Figure 7 shows an ideal situation; in reality, the TCP curve is often shallower than the NTCP curve, partly because tumors are more heterogeneous than normal tissues. In addition to this fact, the TCP curve never reaches a value of 1.0 due to possible microscopic or metastatic spread beyond the primary tumor. To perform a good radiotherapy treatment, it is essential that the mean doses of normal tissues be kept lower than the tumor doses, to minimize the inherent complications of the treatment and to optimize the results [26].

At the beginning of radiation therapy normal tissue cells were thought to be more radioresistant to single doses of radiation than tumor cells. However, it has now been found that both malignant cells and acute response tissue cells have an $^{\alpha}/_{\beta}$ ratio of 10Gy [26].

The term *therapeutic index* describes the tumor response to a fixed level of normal tissue damage, translating the notion of the "cost-benefit" analysis of a radiotherapy treatment. This index varies according to several factors, namely with the dose rate and LET of the

irradiation, with the presence of radiosensitizers or radioprotectors, with the treatment plan and with the precision of administration of the treatment [9, 26].

Relatively to the term therapeutic window this consists of the difference between the dose of tumor control and the dose of tolerance. The larger the therapeutic window the more radiosensitive the tumor, because a smaller dose is needed to obtain the tumor control and a greater dose for the appearance of complications. The lower the therapeutic window, the more radioresistance is the tumor [9].

2.2.4 Biologically Effective Dose (BED)

In 1982, *Barendsen* achieved a major advance in the use of linear quadratic algorithms. The extrapolated tolerance dose (ETD) is defined as a dose administered in infinitely small fraction doses or at an infinitely low dose rate, generating the same cell death. The term ETD was renamed by extrapolated response dose (ERD) when it was concluded that it could be applied to all types of biological effects and not only to tolerance of normal tissues. Subsequently, the term biologically effective dose, BED, was adopted. The term ERD is currently used by Dutch scientists [27, 28].

In 1989 an article was published, by the *British Journal of Radiology*, which introduced the term BED. It is based on the linear quadratic model with the included time factor, substituting terms as the standard nominal dose of 1969. BED is used in iso-effective fraction dose calculations. Over the years, it has undergone several improvements that have allowed its clinical utility to be extended, particularly in the comparison of dose fractionation schemes used in different institutions. When BED calculations are the only data available in a complex treatment, weighting in the analysis of these data is necessary [29].

The term BED represents the cell survival model, is an inherent part of the quadratic linear model and indicates the radiosensitivity of normal or tumor cells to the effect of radiation. The theoretical framework of the model is needed to help understand the term BED, which is explained in Annex A in the section "Appendix A" [29].

BED represents the physical dose required to achieve a given effect if the dose was administered by infinitely small fraction doses or at a very low dose rate in the case of continuous irradiation rates [29].

Using the BED, it is possible to overcome the difficulty in clinical practice of calculating the total dose when a dose change occurs per fraction, through Eq.2 and Eq.3:

$$BED = \frac{E}{\alpha} = \frac{n(\alpha d + \beta d^2)}{\alpha} = nd\left[1 + \frac{d}{\left(\alpha/\beta\right)}\right] \quad (Eq.2)$$

$$BED = D\left[1 + \frac{d}{\left(\alpha/\beta\right)}\right] \quad (Eq.3)$$

Equation 3 represents the most commonly used formula for BED calculations.

BED is the parameter which quantifies the overall biological effect on a given tissue. The equation for BED provides a simple and straightforward way to compare doses from different fractionation schemes, which in turn have different biological effects [29, 30, 31].

For each treatment of radiotherapy performed, there are at least two BED values: that of the tumor and that of the late response tissues. Usually treatments can be compared using as reference the BED values associated with each. BED is useful in the decision process as compensating for interrupted treatment [32].

Based on experimental and theoretical considerations, this model is mechanically plausible for designing protocols in the dose range up to 10Gy/fraction, and, based on animal data, it is reasonable up to 15 to 18Gy per fraction [33].

2.2.4.1 Values of the α/β ratio

The increase in BED values is higher in tissues with low α/β ratios, i.e., it is higher in late response tissues than in acute response tissues [7, 27]. For acute response tissues the α/β ratio is within a range of 7-20Gy, whereas for late response tissues the α/β ratio generally ranges from 0.5-6Gy.

Concerning α/β ratio values for tumor tissues, it should be noted that the values presented by the well-oxygenated head and neck carcinomas and lung carcinomas are identical or slightly higher than the values presented by the acute response tissues. However, there is evidence that some tumors, such as melanomas, sarcomas, early-stage prostate tumors and breast tumors, have low α/β ratios. These may also present

even lower values than those concerning normal late response tissue, namely 0.8-2.5Gy for slow-growing prostate tumors [9, 29].

The $^{\alpha}/_{\beta}$ values for cervical and thoracic cord are 2Gy and 4Gy, respectively. In this way, a dose of 50Gy given in 2Gy daily fractions is equivalent to a BED of 100Gy$_2$ ($^{\alpha}/_{\beta} = 2$) or 75Gy$_4$ ($^{\alpha}/_{\beta} = 4$).

2.2.4.2 Hypofractionation and hyperfractionation

When the dose per fraction is greater than 2Gy it is said that the alternative fractionation scheme is termed as hypofractionation. In this case, BED values decrease more rapidly in late response tissues than in acute response tissues. However, when the dose per fraction is less than 2Gy, we are talking about a case of hyperfractionation where BED values increase more rapidly in late response tissues [9].

Late response tissues are more sensitive to a dose modification by fraction, since the change in the total dose is higher for lower $^{\alpha}/_{\beta}$ ratio values. Hypofractionation can be used as a convenient way of accelerating treatment, i.e., reducing its total time. In some types of tumors, this implementation can lead to short schedules that can be favourably compared with longer schedules in terms of tumor control and late response tissue effects. It is important to note that the advantage associated with a lower fraction dose would be voided for tumors with low $^{\alpha}/_{\beta}$values [9].

2.2.4.3 Dose equivalent in fractions of 2Gy (EQD$_2$)

The approach of the LQ model leads to several formulas that allow us to calculate the isoeffect ratios for radiotherapy. These formulas are intended to describe a set of fractionation schemes that are isoeffect. The simplest method of comparing the efficacy of schemes consisting of different total doses per fraction is to convert each scheme into an equivalent in 2Gy fractions which would give the same biological effect, i.e., EDQ$_2$. The equation that allows the calculation of the equivalent dose in fractions of 2Gy is (Eq.4) [9]:

$$\mathrm{EDQ}_2 = \mathrm{D}\frac{d+(^{\alpha}/_{\beta})}{2+(^{\alpha}/_{\beta})} \quad (Eq.4)$$

Where EDQ_2 represents the dose in fractions of 2Gy which is biologically equivalent to a total dose D with a dose per fraction d. Since EDQ_2 uses doses per fraction of 2Gy, which are the most used in clinical practice, it has the advantage that the values obtained are recognized by radiotherapists [9].

2.2.4.4 Incomplete repair

The simple LQ model states that a time interval between fractions is required to allow full repair of the sublethal damages occurring after each dose of radiation. This time interval between fractions should last for at least 6 hours, but in some cases, such as the spinal cord, it may last up to 1 day. If the time interval between fractions is not performed, in the case of several fractions per day, reparation of fractional damage may not be completed before the next fraction administered. If this occurs, we find that there is an interaction between the unrepaired residual damage of a fraction and the damage caused by a subsequent fraction [9].

When incomplete repair occurs, the dose required to produce the same biological effect decreases. The influence of this repair is determined by the repair interval required for each tissue, which consists of the time needed between fractions for which half of the maximum repair possible occurs. There is an adapted BED formula that considers the situation of the influence of incomplete repair. It is described in Annex A in the section "Appendix A" [9].

2.2.4.5 Time factor – repopulation

Proliferation during radiotherapy treatment decreases the major effects of radiation. In late response tissues, i.e., in tissues with slow proliferation, repopulation is insignificant. However, in tissues with rapid proliferation, such as tumors, repopulation can lead to apoptosis. For tumor repopulation to occur, it is necessary to decrease the size of the tumor so that the cells that are in hypoxia take the place of those that are more at the periphery and then come into contact with the oxygen in the bloodstream [9, 28].

There is a formula for calculating BED considering the repopulation factor, which is described in Annex A in the section "Appendix A".

In most tumors and rapidly proliferating tissues, the repopulation is about 0.5-0.8Gy. In general, human tumors have a doubling time of their volume ranging from 1-3 months,

however the cell doubling rate is 3-5 days. Prostate cancer has an extremely slow proliferation and its cell doubling time is, on average, 42 days [9, 28, 29].

2.2.4.6 Advantages and disadvantages of BED

The use of BED brings advantages such as the comparison of different treatment regimens that allows a posterior evaluation of the existing differences in clinical practice or previous clinical trials. In addition, BED was also used initially in animal experiments, which allowed the preparation of several revisions and the transposition of this concept into clinical practice [28].

In general, the practice of BED has few disadvantages, being increasingly used in radiobiology for comparison of fractionation schemes. A possible disadvantage is the still existing confusion with the biologically equivalent dose, which has long been reiterated as incorrect [28].

Chapter 3

3. The spinal cord

3.1 Anatomy

The nervous system is usually divided into different parts, according to structure and function (Figure 8). Structurally, it is divided into the central nervous system (CNS) and peripheral nervous system (PNS). Functionally, it is divided into somatic and visceral parts [34].

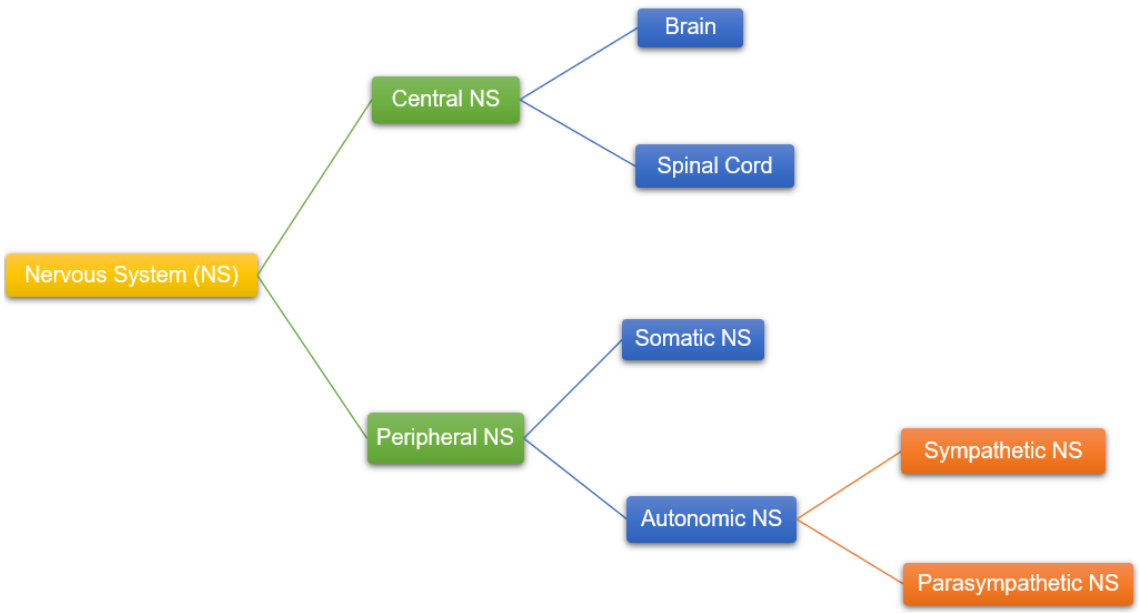


Figure 8 Constituents of the nervous system.

The main functions of the nervous system, which is involved in most of the organic functions, are [35]:

- *Sensory information*, where sensory receptors monitor external and internal stimuli;
- *Integration*, where the sensory information processing organs and response initiators, the brain and the spinal cord, produce an immediate response or store as memory for later use;
- *Homeostasis*, the body's ability to present a constant characteristic physical-chemical situation, where the central, endocrine, excretory, circulatory and respiratory nervous systems are immobilized;
- *Mental activity*, where the encephalon is the centre of mental activities, including consciousness, thought, memory and emotions;
- *Control of muscles and glands*.

The CNS has embryonic origin being composed of the brain and the spinal cord, which are protected by the bones that surround them. On the other hand, the SNP is the part of the nervous system that is outside the CNS. It is composed of spinal and cranial nerves, sensory receptors, ganglia, visceral plexus and enteric system [34, 35].

While the CNS receives sensory information, evaluates it, stores part of it and triggers reactions, the SNP collects information from various sources, from the inside and outside of the body, and relays it through the axons to the CNS. Structural activity develops from the axons of motor neurons in the PNS that relay the CNS information to various parts of the body, especially to the glands and muscles [35].

The encephalon is located inside the cranial cavity and the spinal cord lies within the spinal canal, formed by the vertebrae. The spinal cord is in continuity with the brain through the occipital hole, whose function is to integrate the information received and produce responses through reflex mechanisms (Figure 9) [35].

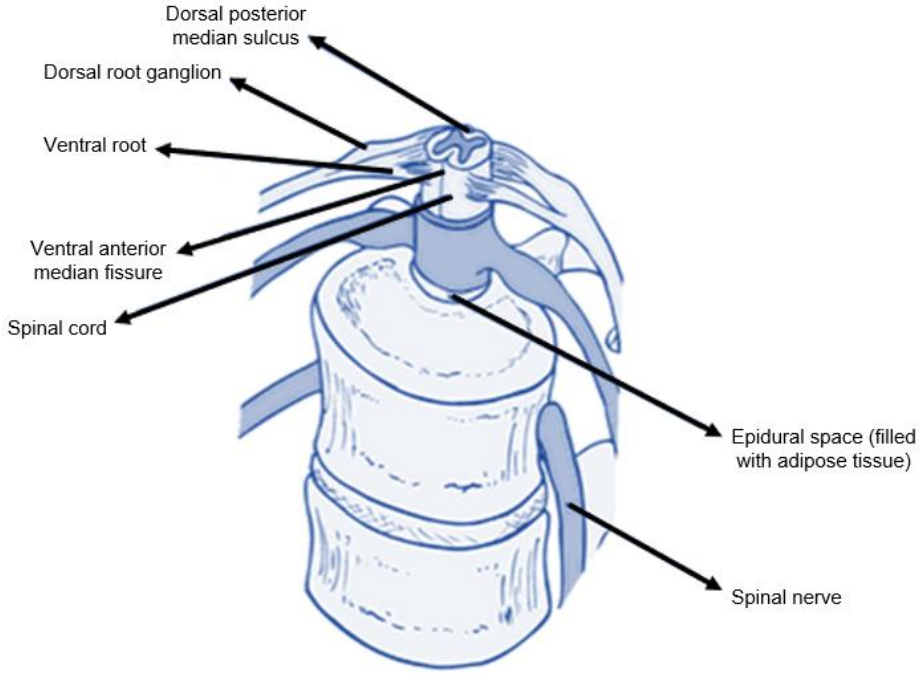


Figure 9 Constituents of the spinal cord. Adapted from [36].

Structurally, the spinal cord extends from the occipital hole to the level of the second lumbar vertebra. This occurs because the spinal growth rate is higher than the spinal cord. It has an approximately cylindrical shape and in the transverse section has a circular/oval shape with a central canal (Figure 10) [34, 35].

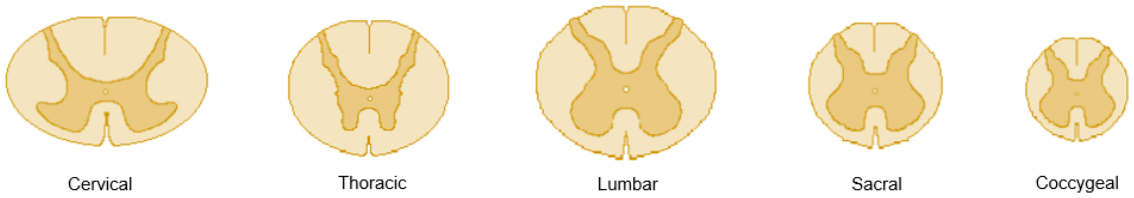


Figure 10 Representation of different sections of the spinal cord.

The spinal cord extends from the foramen magnum to approximately the level of the intervertebral disc of the L1 and L2 vertebrae, in the case of adults. Its length may range and reach only D12, shorter, or reach the intervertebral disc between the longer L2 and L3 vertebrae. In new-borns, spinal cord extension is larger when compared to extension in adults, where it can reach the L3 and L4 vertebrae. The end of the spinal cord is called the medullary cone because it has a cone shape. A terminal filament connective tissue

filament extends from the apex of the medullary cone to the coccyx. This filament is responsible for the attachment of the spinal cord to the coccyx. The medullary cone and the various nerves extending downward are called the equine tail [34, 35].

It is composed of five segments that are designated according to the area of the spine by which their nerves enter and leave (Figure 11) [34, 35]:

- *Cervical segment*, eight pairs of nerves (C1 to C8);
- *Thoracic segment*, twelve pairs of nerves (T1 to T12);
- *Lumbar segment*, five pairs of nerves (L1 to L5);
- *Sacral segment*, five pairs of nerves (S1 to S5);
- *Coccygeal segment*, a pair of nerves (C0).

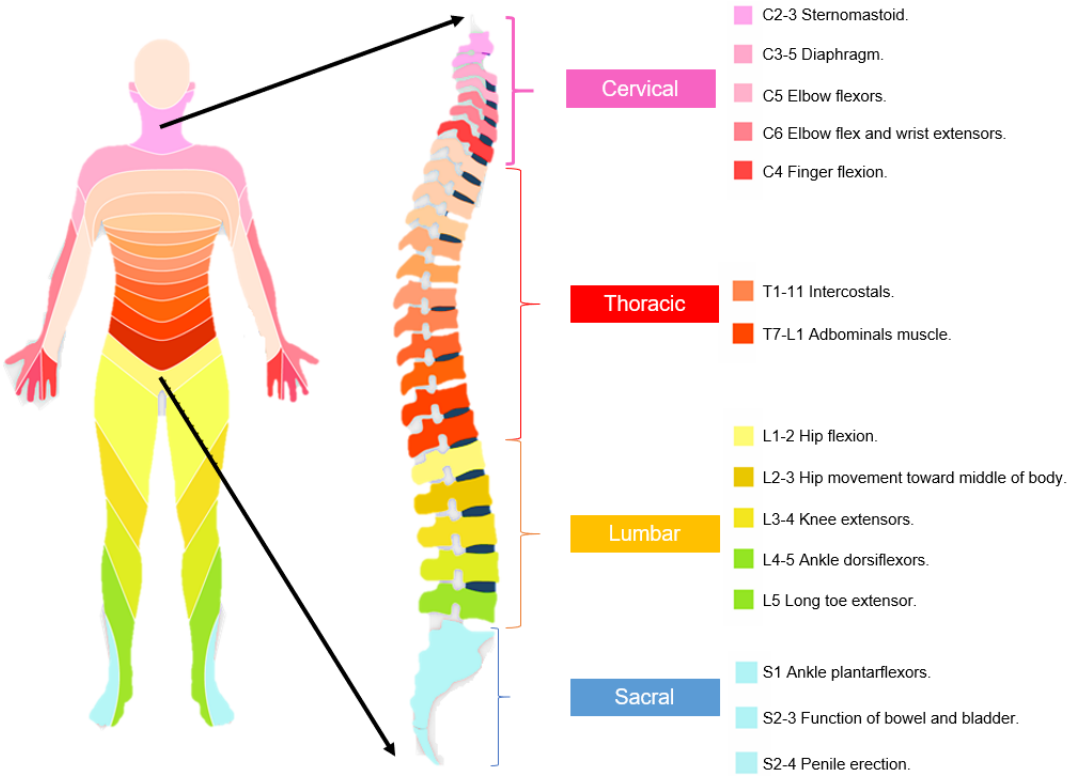


Figure 11 Spinal cord: segments and their function. Adapted from [36].

There are thirty-one pairs of spinal nerves that originate in the spinal cord and pass out of the spine through the intervertebral holes or conjugation holes. The fact that the spinal cord does not have a uniform diameter throughout its length, there is a decrease in the diameter from top to bottom, due to the presence of two dilatations, termed cervical dilatation and lumbar dilatation. These dilations are associated with the origin of the spinal nerves that innervate the upper and lower limbs. Cervical dilatation, responsible for the innervation of the upper limbs, occurs in the region associated with the origin of the spinal nerves from C5 to T1. Lumbar dilation, responsible for innervation of the lower limbs, occurs in the region associated with the origin of the spinal nerves L1 to S3 [34, 35].

Externally, the spinal cord has several grooves and fissures, namely the median anterior cleft, the median posterior sulcus and the posterolateral sulcus. The latter is located on each side of the posterior face and represents the place where the posterior radicular of the spinal nerves enter the spinal cord. In the medullary structure, the grooves are deep crevices that partially separate the two halves of the spinal cord. About the inner surface of the spinal cord, it consists of a central canal surrounded by grey and white matter (Figure 12).

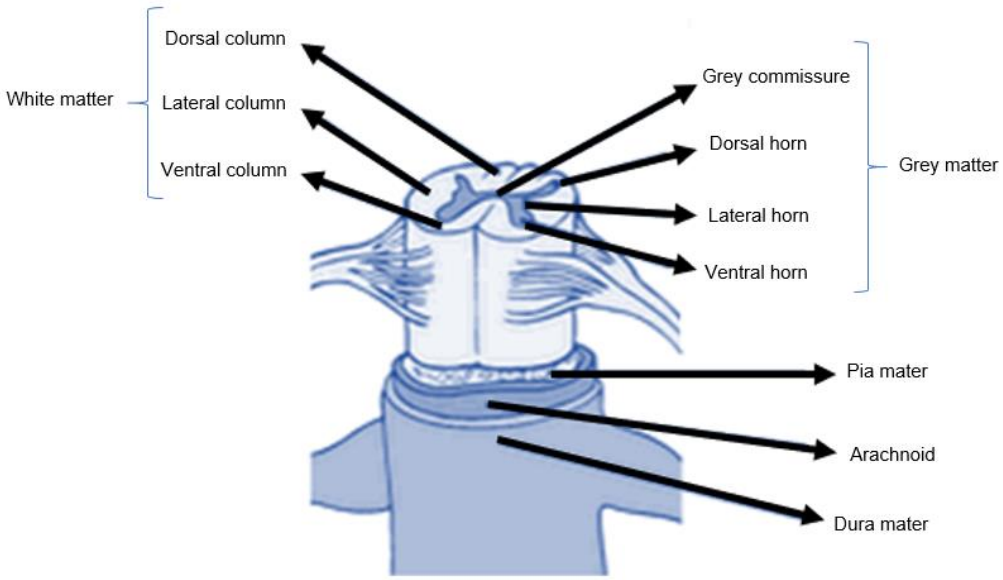


Figure 12 Cross section of the spinal cord Adapted from [36].

The grey matter has numerous bodies of neurons arranged in longitudinal columns along the spinal cord, forming an "H" figure in the cross-sectional images. It has in its constitution axons and dendrites. The grey matter of the spinal cord is arranged in anterior, lateral and posterior horns. On the other hand, the white matter surrounds the grey matter and contains numerous prolongations of neurons, that is, myelinated axons. The white matter is organized on each side of the spinal cord into three strands: anterior, lateral and posterior cord. In turn, each cord divides into bundles or nerve pathways that are responsible for transmitting information to other spinal or brain levels. The two substances of the spinal cord are connected through grey and white commissures. These have axons that cross from side to side of the spinal cord. In the centre of the grey commissure we can find the central canal (Figure 13) [34, 35].

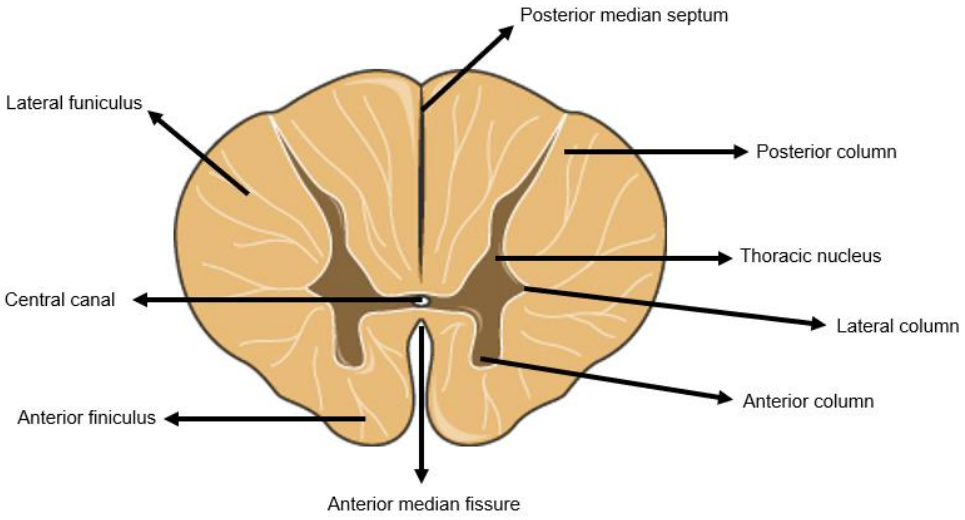


Figure 13 Diagram of transverse section of spinal cord.

The spinal nerves come from numerous radicles that lie along the dorsal and ventral surfaces of the spinal cord. The junction of about six to eight radicles originates each posterior root of the dorsal side. Each of the dorsal roots has a ganglion called the dorsal root ganglion, spinal ganglion and spinal ganglion [35].

The meninges consist of three layers of connective tissue that have the function of enveloping and protecting the brain and spinal cord (Table 6).

Table 6 Types of mater. Adapted from [35].

Types of mater		
DURA MATER	ARACHNOID MATER	PIA MATER
More superficial layer and thicker layer. This layer involves the spinal cord and is in continuity of the epidermis of the spinal nerves. It is separated from the periosteum of the spinal canal by the epidural space. This space has fat, areolar connective tissue and blood vessels.	Very thin and slim layer. The space between this layer and the dura mater is called a subdural space, which contains a small amount of serous fluid.	The pia mater layer bonds very closely to the surface of the brain and spinal cord. In this layer occurs the formation of the terminal filament beyond the medullary cone. The space between the arachnoid layer and the pia mater is called the subarachnoid space containing encephaloraquidian fluid.

The last topic to mention is the vascularization of the spinal cord. The arteries that vascularize the spinal cord region are anterior and posterior root arteries. There are also segmental medullary arteries and anterior and posterior spinal arteries. The veins that drain the spinal cord form some longitudinal channels: two pairs of veins on each side supporting the connections of the anterior and posterior roots of the spinal cord, a midline channel that is parallel to the anterior medial cleft and a midline channel that follows along the posterior median sulcus [34].

3.2 Physiology

The spinal cord represents an important role in mediating simple reflexes and in creating coordinated sequences of movements. According to *Sten Grillner*, in all vertebrates the spinal cord produces a basic pattern of locomotion coordination, whether it is movements that are required to walk, swim or fly [38].

The PNS contains twelve pairs of cranial nerves, in addition to the thirty-one pairs of existing spinal nerves. The spinal nerves are designated according to the region of their exit (Table 7).

A nerve consists of nerve fibers consisting of axons of afferent neurons, afferent neurons, or both. Thus, the fibers of the nerves can be divided into two groups: afferent division or efferent division of the PNS. Afferent fibers are responsible for conducting information from the sensory receptors of the PNS to the CNS.

Table 7 Description of the functions of the different spinal nerves. Adapted from [39].

Spinal nerves	Function
Cervical	Control of muscles and glands, receiving sensory information from the head, neck, arms and hands.
Thoracic	Innervation of the thoracic and abdominal walls.
Lumbar	Associated with the hip and legs.
Sacral	Innervation of genitals and lower digestive tract.
Coccygeal	Innervation of the coccyx.

The efferent division is subdivided into the somatic nervous system and the autonomic nervous system. Somatic division neurons innervate the skeletal muscles, while autonomic division neurons innervate the smooth and cardiac muscles, glands and neurons of the gastrointestinal tract [39].

In the dorsal roots, from the peripheral nerves, groups of afferent nerve fibers enter the spinal cord. The axons of the efferent neurons leave the spinal cord through the ventral roots. At a small distance from the spinal cord, the dorsal and ventral roots of the same level join to form a spinal nerve on each side of the spinal cord [39].

3.3 Tumors in the spinal cord

A tumor located inside or near the spinal cord can stop communication between the bundles, which transmit messages between the brain and nerves throughout the body and threaten the patient's health [40].

The tumors formed in this organ develop between the protective sheaths or the superficial sheath that lines the spinal cord.

Most tumors that develop inside the spinal cord do not progress to other parts of the body being termed primary. Generally, they are benign tumors. On the other hand, malignant spinal cord tumors are secondary tumors, that is, they spread through the body from a primary cancer located elsewhere in the body.

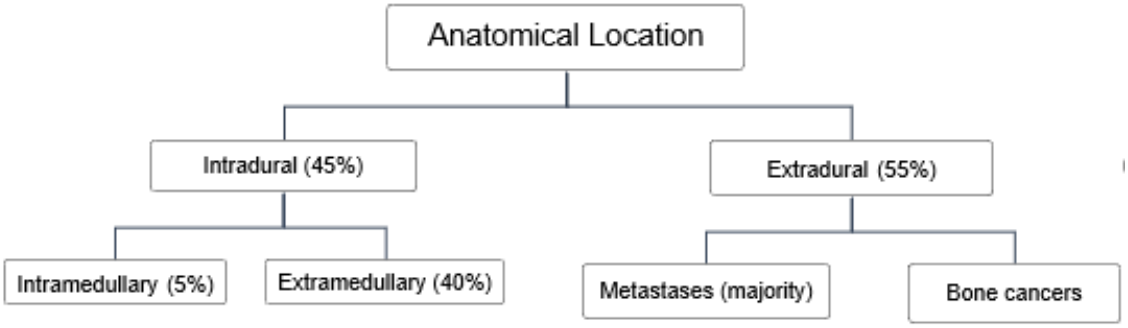
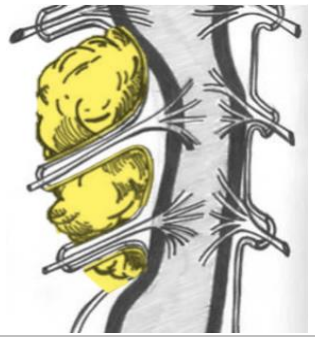
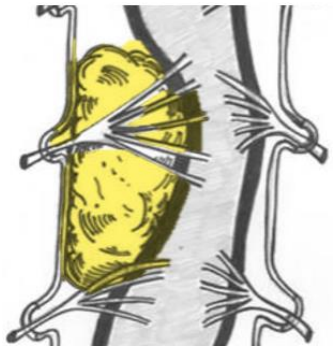
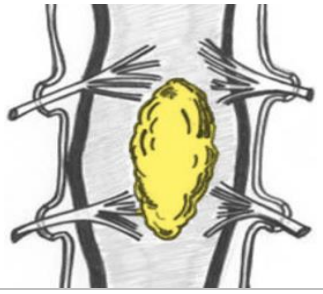


Figure 14 Representative scheme of the type of tumors in the vertebral column.

Of the tumors found in the spine, 95% are not found in the spinal cord: 55% are in the vertebrae or in the circulating tissues outside the dura mater, and 40% are in the dura mater. The remaining 5% appear in the spinal cord (Figure 14). Spinal cord tumors are usually divided according to their location (Table 8).

Table 8 Description of the types of tumors that may arise in the spinal cord. Adapted from [41, 42].

Tumor	Description	Visualization
Extradural	Proliferate outside the dura mater and are usually located in the vertebrae. They are often secondary malignant tumors, i.e., metastases.	
Intradural extramedullary	They develop within the dura, outside the spinal cord. Usually, these tumors are benign and proliferate slowly. Most of these spinal tumors are: meningiomas (occurring in the membranes that surround the spinal cord and are usually benign but may be malignant, tumors are more common in middle age and in older women) and tumors in the nerve sheath.	
Intramedullary	They grow inside the spinal cord. They are often benign primary tumors.	

3.4 Complications after irradiation in the spinal cord

The spinal cord is the most dose-limiting organ in radiation therapy. In this organ, radiation-induced lesions can result in devastating functional deficits that may manifest in months or years after radiotherapy treatment [5].

Advances in biology, physics and radiotherapy have led to an evolution in the treatment of tumors and consequently survival rates have increased for a wide variety of malignancies.

However, this increase in survival has consequences that need to be considered. Patients who have already suffered from a malignant disease are at increased risk of developing a second neoplasm. There are three main reasons for this [9].

1) When comparing individuals with the same lifestyle, age, gender, and other characteristics, we can observe that patients with a history of neoplastic disease are at greater risk of developing a second neoplasm;
2) The etiological factors, related to the first tumor, can remain active and promote the evolution of a second neoplasm;
3) The therapies used to combat the first tumor, namely chemotherapy and radiotherapy, are associated with an increased risk of developing second neoplasms.

In addressing the issue of irradiation of normal tissues it is necessary to consider account two concepts: acute toxicity and late toxicity. Acute toxicity affects proliferative and repairing tissues such as the epidermis and mucous membranes. Damage repair is performed using surviving stem cells that are within the irradiated volume or based on the stem cells that migrate from the non-irradiated sites to the irradiated sites. These cells allow regeneration and restoration of tissues and cells resulting in complete or partial restoration of radiation tolerance [9].

Despite the advances made, acute toxicity continues to have a significant effect on patients' quality of life. Some of the acute responses from radiation therapy are dose limiting, such as oral mucositis in advanced head and neck tumor radiotherapy [9].

On the other hand, late toxicity results in parenchymal damage that may lead to eventual loss of function within the irradiated volume. The clinical consequences of irradiation depend on the organ architecture and irradiated volume. It is essential to note that the radiosensitivity of a single cellular component cannot be used as a predictor of organ sensitivity. In the case of re-irradiation, for palliative purposes, late toxicity may not

become clinically apparent during the limited life span of most patients. In the case of re-irradiation, it is necessary to evaluate the biologically effective dose (BED) and the delayed effects that come from the previous treatment, and it is necessary to exclude the patients who tolerated the previous treatment [6, 9].

It is necessary to take into consideration the irradiation of the spinal cord when irradiating areas such as the abdomen, pelvis and in cases of bone metastasis in the spine, this occurs in about 40% of the patients with cancer [43].

Several authors described the effects felt in the spinal cord after irradiation, although the definitions and concepts may differ between them. According to *Rega et al.*, one of the complications of the spinal cord is radiogenic myelitis that can be defined as exclusively chronic radiogenic myelopathy syndrome. This syndrome manifests itself between 9 and 15 months and may also manifest up to 3 years after irradiation and is associated with symptoms such as paraesthesia, sensory disorders and, sometimes, dysfunctions in the bladder and intestine with late development [44, 45].

There are several clinical syndromes of radiogenic myelopathy:

- The most common myelopathy, which is not associated with any other abnormality in the neurological examination, is characterized by the presence of the *Lhermitte's* signal. This signal consists of a sensation of shocks that travel through the cervical and thoracic segments of the spinal cord with irradiation to the upper limbs and sometimes to the lower limbs when the patient performs flexion of the cervical spine [43]; Acute transient radiogenic myelopathy occurs most commonly in the cervical and thoracic segments of the spinal cord, and it is estimated that approximately 15% of patients with mantle irradiation in *Hodgkin's* disease develop this syndrome. Transient demyelination occurs due to a lesion in the oligodendrocytes. These cells are responsible for the formation and maintenance of the myelin sheaths of the CNS axons [44, 45];
- A possible syndrome is secondary to a spinal cord injury caused by damage to the blood vessels caused by radiation [44, 45];
- The third syndrome results, presumably from selective damage to the anterior horn of the grey matter, and this part of the bone marrow contains motor neurons responsible for axial movements [44, 45];
- Finally, chronic radiogenic progressive myelopathy where pathological findings have been described and is associated with permanent signs that are caused by vascular damage and damage to oligodendrocytes. These damages result in necrosis of the white matter or in demyelination. However, there are patients who

stabilize after a partial neurological loss, this event is called Brown-Sequard Syndrome, characterized by a classic presentation that combines sensory motor and tactile deficits, vibration, sensory deficits of the joint position on one side of the body and sensory deficits in temperature and pain on the contralateral side. This syndrome is most commonly referred to in radio-oncology. It is progressive and permanent and often leads to fatal complications such as an infection or pulmonary embolism. Chronic progressive myelopathy has associated risk factors such as: the total dose and dose per fraction administered, the volume and segment of the spinal cord irradiated and the re-irradiation of the marrow for the control of the malignant disease. All these factors have an indefinite relative influence regarding the dose given incidentally to the spinal cord, when treating tumors in their anatomic region [44, 45].

Spinal haemorrhage is also a late complication that can occur in the spinal cord after its irradiation. It can occur 6 to 30 years after its irradiation, being rarely reported. It develops within irradiated sites, but outside the location of the primary tumor. The symptoms that the patients initially present are: weakness in the lower limbs and painful complaints in the back. These symptoms progress rapidly to paresis and tetraparesis. Telangiectasias caused by radiation are the probable cause of these haemorrhages [44].

The lesions that occur in the spinal cord are divided into three groups (Table 9).

Table 9 Lesions in the spinal cord appear in different ways after irradiation. Adapted from: [46].

Lesions in the spinal cord	Description
Early	There are no record of acute central nervous system (CNS) syndrome after large single doses to the cord. Clinically, it was found that the damage that occurred is generally related to increased tumor edema as in the context of extradural cord compression.
Early delayed	L'hermitte syndrome is observed after doses well below the threshold of myelopathy, and it is not associated with permanent myelopathy. this state occurs after a latent period of 2 to 4 months and is characterized by paraesthesia in the back and extremities upon neck flexion typically, followed by complete clinical recovery after a few months. It is characterized by an electrical sensation that runs down the back and into the limbs. The sensation can feel like it goes up or down the spine and it is uncomfortable for the patient.
Late	RM is a typical late affect. This disease is normally irreversible. After the diagnosis of permanent myelopathy, the median survival of the patients was 8 months. The latent time after a single treatment was 18 months and after re-irradiation was 11 months.

3.5 Spinal cord doses and tolerance

As previously mentioned, there are several factors that are associated with the development of radiogenic myelopathy. These factors combined with the remaining complications associated with spinal cord irradiation are profoundly related to the tolerance of this organ to radiation.

Tolerance is an essential concept when treating radiotherapy because only through its knowledge can it be possible to plan treatments that do not compromise the integrity of healthy organs and allow the lowest risk of complications.

The dose of radiation that can be delivered for tumor control is limited by late and generally irreversible injury of the surrounding normal tissues and organs at risk (OAR), also known as late effects.

The spinal cord is the most critical organ at risk. It is typically located near the vertebral tumor target volume and has been classically described as an organ with a serial functional architecture and as such damage to small volumes within the structure can have a major impact on neurologic function [47].

As a result of greater accuracy and effectiveness of cancer treatment, patient survival rates increase, and radiation oncologists are frequently faced with the problem of treatment of local recurrence or second tumors located within or close to previously treated sites [32].

Radiation myelopathy is one of the most devastating complications of clinical radiotherapy resulting in severe and irreversible morbidity. Assessment of the impact of dose and fractionation schemes on tissue tolerance has been a major area of research in radiation oncology.

A comprehensive set of dose tolerance limits for normal tissue to RT became a reference landmark in radiation oncology [48]. In an extensive review of spinal cord re-irradiation, it was concluded that a dose of 50Gy in the spinal cord causes a risk of myelopathy of 0.2%. If the dose increases to 59Gy the risk of myelopathy increases to 5% [45, 49].

There is consensus regarding the dose of tolerance accepted in the spinal cord that with conventional fractionation of 2Gy per day including the full cord cross-section, a total dose of 50Gy, 60Gy and 69Gy is associated with a 0.2, 6 and 50% rate of myelopathy [50].

As a result, standard RT involving spinal cord treatment delivers a maximum dose of 50Gy with conventional fractionation 1.8-2Gy per fraction. Data obtained experimentally indicates that dose reduction per fraction below 2Gy does not significantly alter the absolute dose-response [45].

3.6 Radiobiology of the spinal cord

The various outcomes associated with spinal cord irradiation are vascular damage, radiogenic myelopathy and white matter necrosis. The main factors related to the development of myelopathy are related to [9, 29, 45, 51]:

- Total administered dose;
- Dose per fraction;
- Volume and irradiated segment of the spinal cord;
- Re-irradiation of the spinal cord due to the need to control tumor disease;
- Variability of radiation sensitivity between different patients.

When the location of the tumor is very close to a critical organ, such as the spinal cord, the dose it receives will not be homogenous throughout its length. Although this situation is recurrent, the influence of inhomogeneous distribution on bone marrow tolerance is not known. This is because existing data describing the dose-response relationship and dose-volume effects of the central nervous system are based on animal experiments following homogenous spinal cord irradiation [52, 53].

In rats, the grey matter does not present any damages with doses up to 80Gy. On the other hand, the white matter in the left lateral half of the cervical cord was more sensitive than the white substance located in the middle. Necrosis of the white matter results in paralysis [53].

There is a difference in radiosensitivity between the grey and white matter that probably originates from the anatomical and physiological differences in the different parts of the spinal cord, since the supply and vascular density are distinct in these two areas. Regionally, vascular density is greater in the grey matter than in the white matter, being greater in the ventral half than in the dorsal half of the grey matter. The proportion of blood flow from the grey matter to the white matter is 3:1. The blood flow of the white matter has a non-homogeneous pattern and the grey matter pattern is more variable. Thus, the regional difference in white matter radiosensitivity is not only associated with

vascular damage because there is no regional difference in vascular density or blood flow [43, 53].

The grey matter consists mostly of whole cells, while the white matter contains axons and myelin sheaths. The myelin sheaths have an insulating function, in a purely speculative way, it can be said that this structure can be damaged by radiation and thus conclude that the white matter is more radiosensitive. Along the extension of the spinal cord the amount of white matter decreases because there are fewer axons. Therefore, the greater sensitivity of the white matter may be associated with the presence of *Schwann* cells, which accumulate myelin, allowing to conclude that a spinal region is more sensitive the greater the amount of white matter [51].

Although a matter of great importance, radiosensitivity of the spinal cord was, until a few years ago, a subject ignored by radiation oncologists. A non-significant part of radiation oncologists considered the different doses of tolerance along the spinal cord and the effect of volume also did not reveal a major weight in medical treatment decisions. A reason for this fact is related to the scarcity of clinical data on the subject in 1998 [54].

Chapter 4

4. Re-irradiation

Due to the increased survival rate of patients with multiple tumors, there are a greater number of cases where radiation oncologists are faced with the need to treat late recurrences or second tumors. Radiotherapy is one of the possible treatments to combat second primary tumors or recurrences and, for this, it is necessary to consider certain parameters in the decision making of a re-irradiation. This decision becomes complicated because these recurrences and new tumors are located within or near the previously treated region. Tolerance doses of adjacent organs cause concern and need special attention. The tolerance dose of normal tissues is significantly reduced in re-irradiation when compared to that of the first treatment [5, 6].

When assessing tumor re-irradiation, it is necessary to consider whether the dose of tolerance of an organ has already been reached in the first course of treatment. Treating an area that had prior radiation is far more complex than the initial treatment.

When tissue tolerance is not achieved and only induction of subclinical or minimal damage occurs in the long-term recovery or induction of residual damage with manifestation after long years, re-irradiation may be considered. Re-irradiation has a curative and palliative purpose. In case of re-irradiation with curative intent it is necessary to evaluate the biologically effective dose and the late effects of the initial treatment. On the other hand, in the palliative re-irradiation the late toxicity felt may not manifest during the limited life of the patient [6, 9].

In re-irradiation it is of extreme interest to consider the knowledge about the kinetics of the evolution of the hidden damages caused by the previous treatment. Key re-irradiation parameters evaluated in the decision process include [5, 55]:

- Data from previous treatment such as irradiated spinal cord volume and region;
- Total dose;
- Dose per fraction administered;

- Time elapsed since the first treatment;
- Additional treatments for the first tumor (*e.g.* chemotherapy, 'biologicals');
- Organs and tissues involved;
- Alternative treatment options.

Over the years the treatment administration has become more accurate and safer due to the appearance of equipment and techniques, and an evolution of radiation oncology has been observed. The possibility of performing a re-irradiation treatment, where the organs of risk can receive high cumulative doses of radiation, appear due to new knowledge in radiotherapy that allow the approach of new possibilities of treatment [6].

Despite the remarkable development in RT, it is necessary to consider the clinical side effects that may result from a re-irradiation. It is essential to give importance to certain aspects such as: the possibility of disease progression in other parts of the body, the general state of the patient, the acute and late toxicities caused by the treatment, as well as the quality of life of the patient [6].

Many radiation oncologists rejected re-irradiation for curative purposes, after a radical radiotherapy treatment, due to the complications inherent in this type of treatment. In the literature, several studies began to emerge with experimental data indicating the recovery capacity of previously caused damages of some tissues. Radiation oncologists, being aware of these studies, remained reluctant to re-irradiate patients who had previously received high doses. This reluctance prevented re-irradiation for the curative and palliative purposes of some patients [55].

There are two factors that benefit the re-irradiation process [56]:

- Low doses administered on the first treatment;
- Long-time interval between treatments.

4.1 Re-irradiation of spinal cord

The spinal cord is the most critical organ at risk (OAR). This organ is typically located near the vertebral tumour target volume and has been classically described as an organ with a serial functional architecture and as such damage to small volumes within the structure can have a major impact on neurologic function [47].

Myelopathy is a devastating late-effect of radiation therapy, and the spinal cord is considered one of the most critical dose-limiting organs. For this reason, radiation-

induced myelopathy is one of the most feared complications associated with SBRT given that patients may become paralyzed and even die [40, 46, 57, 58].

When the spinal cord is within the target volume, physicians choose for more conservative treatments since radiogenic myelopathy is a problematic late consequence. However, this greater conservatism at the time of the treatment may result in a dose to the sub-optimal tumor which will give rise to a reduced probability of tumor control. In several clinical cases it is necessary to consider if a higher dose in the tumor compensates the increased risk of developing myelopathy. There are rare situations where the development of this pathology occurs below a dose of 45Gy, this fact is due to three factors [5, 32, 45]:

1) *Extrinsic factors*, which reduce radiation tolerance in some individuals;
2) *Thousands of people are irradiated annually with this dose*, which increases the probability of occurrence of these rarer situations;
3) *Actual doses given are higher than the estimated doses*.

Re-treatment in periods shorter than two years may increase the risk of developing complications, since they are more likely to express themselves during this time period [45].

For a total dose of 34Gy to the spinal cord, it is estimated that after 1 year recovery is 76%. For a total dose of 38Gy it is estimated that after 2 years, a recovery of 85% occurs [43].

Initial dose influences different time intervals from tissue tolerance to re-irradiation as well as conditioning the recovery of radiation damage in the first treatment. It is possible to administer a higher dose in the re-irradiation if smaller doses were used at the first treatment and if the intervals between treatments were longer [5, 32].

The occurrence of myelopathy should not be reduced by the limitation imposed on the total doses administered. This reduction should result from determining factors such as: the patient's physical state and the average history, which give rise to a greater radiosensitivity of the spinal cord. If this is justified, the risk of developing myelopathy may be overlap by a significantly greater hypothesis of tumor control [45, 56].

4.1.1 Time interval between fractions / total treatment time

All decisions regarding re-treatment of the spinal cord have been performed to date, based on empirical data. However, in the experimental literature there are several evidences that corroborate the capacity of recovery of the spinal cord depends on the time elapsed between the two treatments. Although there is evidence that proves the recovery of the spinal cord, there are still some controversies in the transformation of experimental data obtained in animals for clinical situations. These controversies stem from the differences between the two groups of living beings [32].

In fractional schemes with multiple daily fractions it is essential to create a break between fractions as long as possible. Due to the catastrophic effect of radiogenic myelopathy, repair of sublethal damage in the spinal cord may not be complete after an interval of 8 hours. The kinetics of repair of the spinal cord is slower, so it is not advantageous to use schemes with interfraction intervals of less than 6 hours. When the tissue repair interval exceeds 4 hours, even doubling the interval time, i.e., 8 hours, there will still be no amount of damage that has not been repaired [32].

A significant recovery of the long-term damage was shown to occur in the rat spinal cord. The tolerance of the organ to the re-irradiation was influenced by the level of initial damages, as well as, the time of expression of the damages occurred (latency time). Thus, increasing the time interval between treatments results in an increase in recovery from damage and, consequently, a recovery of what is generally estimated, leading to prescribed doses in re-treatment that are lower than necessary [5,32, 43].

It was demonstrated that the latency time for paralysis increases with the increase in the time interval between initial treatment and re-irradiation, decreasing with increasing of the damage. The latency time for myelopathy decreases after the required re-irradiation [5, 32].

Time of recovery from damage depends on the tissue or species, as well as on the age of the individual: In animal experiments performed it was observed that 3-week-old mice showed effects faster than adult mice. However, long-term recovery occurred more rapidly in younger rats (3 weeks old) [32, 43].

Long-term recovery was observed after 8 weeks and it increased with time interval to re-treatment. The initial dose influenced tolerance to re-treatment and radiation damage repair at different intervals with long-term recovery after 6 months being approximately 45% [5, 32].

This evidence shows that, the younger, immature spinal cords are slightly more susceptible to radiation and have a shorter latency period. Thus, special care is required in spinal cord irradiation in pediatric situations because of the increased sensitivity of the child to radiation because their CNS and skeleton are in development [43].

In conclusion: re-treatment in periods shorter than two years may increase the risk of developing complications, since lesions are more likely to develop during this time period [43]. Nevertheless, when the time interval between the first treatment and the re-treatment is less than 6 months; the biologically effective dose of each treatment is less of 98Gy_2, no cases of radiation myelopathy where observed when cumulative BED is ≤120Gy_2 [31].

4.1.2 Fractionation

During the 20th century, radiobiological investigation revealed that fractionation of a dose of radiation often produces better tumor control for a certain level of toxicity of normal tissues. Adopting dose fractionation, instead of administering the total dose of radiation in a single fraction, better preservation of normal tissues is achieved which may result from repair of sublethal damage between fractions. The emergence of new alternative fractionation schemes in clinical radiotherapy, which lead to the development of new treatment strategies, results from radiobiological research and clinical observations (Table 10) [6, 32].

Table 10 Different types of fractionation schemes and their description. Adapted from: [7].

Types of fractionation	Dose per fraction	Time of administration	Weekly dose
Accelerated	1.8–2.0Gy/day	More than 1 fraction/day	–
Conventional	1.8–2.0Gy/day	5 days/week	9.0–10.0Gy
Hyperfractionation	< 2.0Gy/day	2 fractions/day, with interval of 6–8 hours	–
Hypofractionation	> 2.0Gy/day	< 5 days/week	–
Continuous accelerated hyperfractionated radiotherapy (CHART)	–	3 fractions/day, during 12 days with intervals between fractions of 6 hours	–

The fractionation schemes that administer more than a daily fraction are: hyperfractionation and accelerated fractionation, in which in these types of fractionation the interval of time interferes changes the 24 hours for a range of 3 to hours, depending on the scheme used. Thus, a devaluation of the benefit of the treatment may occur since

the achieved tumor control does not compensate for the exceeded tolerance dose of the tissues. This excess tolerance dose originates from the incomplete repair of damage between fractions [32].

When radiotherapy is administered for curative purposes, there is a greater risk of developing lesions in normal tissues. Thus, it is necessary to know the rate of repair of the tissues to later select the time interval between fractions and the most appropriate fractionation. The rate of tumor control depends on the tolerance of normal tissues to radiation. Clinical data on spinal cord tolerances to re-irradiation are rare and generally come from reports of limited cases [32].

It is possible to conclude that initial doses influence [5]:

- Different time intervals from tissue tolerance to re-irradiation;
- Recovery of induced damage in the first treatment.

It is possible to administer a higher dose in the re-irradiation if smaller doses were used at the first treatment and if the intervals between treatments were longer [5, 32].

To obtain a better fractionation scheme it is important to obtain radiobiological data with the purpose of confirming or refuting the biological models. If excessive risk factors are controlled, it is possible to maintain the level of development of myelopathies at a minimum, making the doses prescribed to tumor volume the most appropriate for the disease [32].

4.2 Stereotactic body radiotherapy (SBRT)

Initially, this technique was developed for the treatment of spinal metastases when the clinical setting was a re-irradiation. However, it is currently an emerging treatment option for vertebral metastases with proven efficacy in the initial, post-operative, re-treatment, and for tumors with radioresistant histology [47, 59, 60].

SBRT is a new method of treatment in radiotherapy, which provides a high dose of high radiation to small and well-defined targets with a single or a few fractions with a high degree of precision inside the patient's body. This type of therapeutic protocol is called hypofractionation of radiotherapy and is characterized by its high biological efficacy, with favourable clinical results about tumor control and the rate of late complications [58]. SBRT uses radiation doses in a few fractions, usually 1 to 5, for the target, thus, spinal cord tumors are treated with high biologically effective doses (BED).

According to the American College of Radiology (ACR) and the American Society for Radiation Oncology (ASTRO), SBRT is define as “an external beam radiation therapy method used to very precisely deliver a high dose of radiation to an extracranial target within the body, using either a single dose or a small number of fractions.” [61].

More and more cancer patients are treated with SBRT. The change from conventional fractionated radiotherapy to hypofractionated SBRT is due to technological advances in tumor imaging and radiation delivery systems [62].

This treatment involves a high level of precision, accuracy and reproducibility throughout the treatment process. To deliver radiation only at the target volume, it is necessary to minimize the margins for dose delivery. For this, the location of the target volume and its movement is limited. Patient immobilization systems must be very accurate, and the radiation equipment must have a mechanical tolerance for emission of +/- 2mm [58, 63]. In the case of the IPO-PORTO, this mechanical tolerance for emission is less than 1mm.

To minimize normal tissue toxicity, conformation of high target doses, and rapid dose drop gradients off the target, are obtained through multiple coplanar, non-coplanar static fields or arc therapy [58].

Flattening filter free (FFF) linear accelerators allow for an increase in instantaneous dose-rate of the x-ray pulses by a factor of 2–6 over the conventional flattened output As a result, radiobiological investigations are being carried out to determine the effect of these higher dose-rates on cell response [64]. The studies reported thus far have presented conflicting results, with some suggesting that increasing dose rate is an acceptable manner of decreasing radiotherapy treatment time that does not have any detrimental radiobiological effects [65].

Compared to conventional fractional radiotherapy, where large volumes of normal tissues are irradiated, the main goal of SBRT is to reach the tumor, avoiding irradiation of adjacent normal tissue (Figure 15). SBRT has specific characteristics that differentiate it from conventional RT [58, 66]:

- *Treatment scheme*, where dose delivery is performed in one or a up to 5 fractions, resulting in a high BED value. In several studies, we can verify that a re-irradiation performed with a SBRT treatment has higher BED values when compared to the first conventional radiotherapy treatment (Table 11);

Table 11 Variation in BED value according to treatment schedule.

		Initial treatment		Re-irradiation treatment	
Author	α/β value	Dose fx(Gy)/no fx	BED	Dose fx(Gy)/no fx	BED
Grosu *et al*. [67]	2	1.25/32	65.0	1.8/16	55.1
		1.4/20	49.4	3.0/10	75.0
Wright *et al*. [68]	4	3.0/10	53.0	6/5	26.0
	4	3.0/15	79.0	6/5	15.0
	2	3.0/11	83.0	6/5	20.0
Sahgal *et al*. [40]	2	1.8/30	102.6	8.0/3	120.0
		0.9/28	36.5	10.5/2	131.3
		2.88/15	105.4	16.0/1	144.0

- *Small irradiated volumes*;
- *Inhomogeneous dose distribution* (the inherent sensitivity of the dose distribution secondary to the steep dose gradient beyond the target volume);
- *Short treatment time* (in a conventional treatment since the duration of the treatment would last for several months).

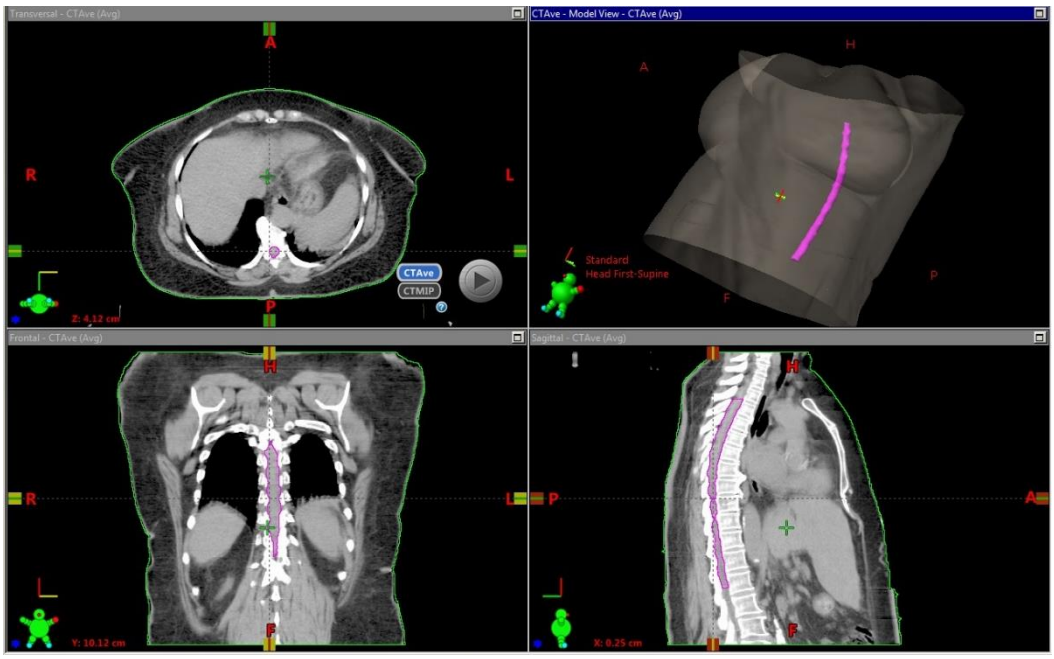


Figure 15 Example of contour of the spinal cord. (Images provided by the Medical Physics Service of the IPO-Porto).

In the limits defined by Emami [48, 49] all prescriptions were in 1.8 or 2Gy fractions. In contrast, SBRT prescription schemes typically range from 5Gy per fraction up to 20Gy per fraction or more. In this new radiation delivery paradigm, normal organ dose tolerance limits and the dose-volume response of the tumors depend strongly on the number of fractions used and the dose per fraction.

After more than 20 years of Radiation Therapy Oncology Group (RTOG) trials, conventional radiation therapy had progressed to the point where it is possible to define dose tolerance limits in terms of 5% or 50% chance of a specified adverse event occurring within five years. In contrast, published follow-up data for SBRT is inadequate to reliably determine the probability of adverse events [63].

4.2.1 Late effects

The occurrence of normal tissue damage may be associated with the amount of dose that is delivered in each fraction, the duration of treatment, the areas of the nervous system that are irradiated, the total radiation received by the patient and the patient's susceptibility to radiation. Symptoms of these damages can manifest themselves in the first days of the treatment, called early effects, or months after the last irradiation, late effects [66].

When compared to conventional fractional radiotherapy, hypofractionation, used in SBRT, is more likely to cause late effects, i.e., late radiation injury.

Late effects in adjacent normal tissues are more difficult to treat when compared to early effects. Late vascular injury can make tissues less viable, having a low blood supply, poor healing, poor functional capacity and lead to necrosis, resulting in problems such as fistulas and ulcers, which can be debilitating and even deadly [66].

Hypofractionation, in 1980’s and 1990’s, was not accepted as a form of radiotherapy treatment, since it was proved that it caused more delayed effects than conventional fractionation, when the same total dose was administered. Thus, only patients with a very limited life span were treated with hypofractionation, as it was believed that they would not live long enough to present long-term effects [66].

Advances in biology and physics have allowed increased precision and accuracy in radiotherapy in order to maximize tumor damage and to minimize lesions in the dose limiting adjacent normal tissues. This led to an evolution in the treatment of tumors and consequently survival rates have been increasing for a wide variety of malignancies.

Spine SBRT, also known as spine stereotactic radiosurgery (SRS), is an emerging treatment option for patients with spinal bone metastases with or without a soft tissue component and is rapidly being adopted in the clinical although with limited high-quality evidence [47, 63].

SBRT is a highly effective treatment able to deliver ablative doses to tumors with minimal doses to the surrounding normal tissues. It Is defined as radiotherapy treatment of tumors outside the brain with 1 to 5 dose fractions. It has been made possible in recent years due to advances in image guidance, target visualization and radiation delivery methods that allow large doses delivered to the target while sparing critical organs at risk (OARs).

Outstanding results already have led to the suggestion that high doses per fraction may have greater efficacy than conventional fractionated radiation therapy [25]. Local control rates in spine re-irradiation with SBRT reported in the literature range from 66% to 92% at 1 year [70].

Initially, this technique was used to successfully palliate spinal metastases, even for tumors of radio-resistant histologies. This success with spinal metastases has led to the widespread extrapolation of SRS to the treatment of primary spine tumors and for benign tumors of the spinal cord [47, 59].

Radiation myelopathy (RM) is a late effect of radiation treatment delivered to the spinal cord. It is a diagnosis of exclusion, based on neurologic signs and symptoms consistent with damage to the irradiated segment of the spinal. This disease is a relatively rare disorder characterized by white matter lesions of the spinal cord resulting from irradiation. The observed clinical effects may be related to sensory and/or motor deficits to complete paraplegia/quadriplegia and loss of autonomic functioning (Figure 16) [47].

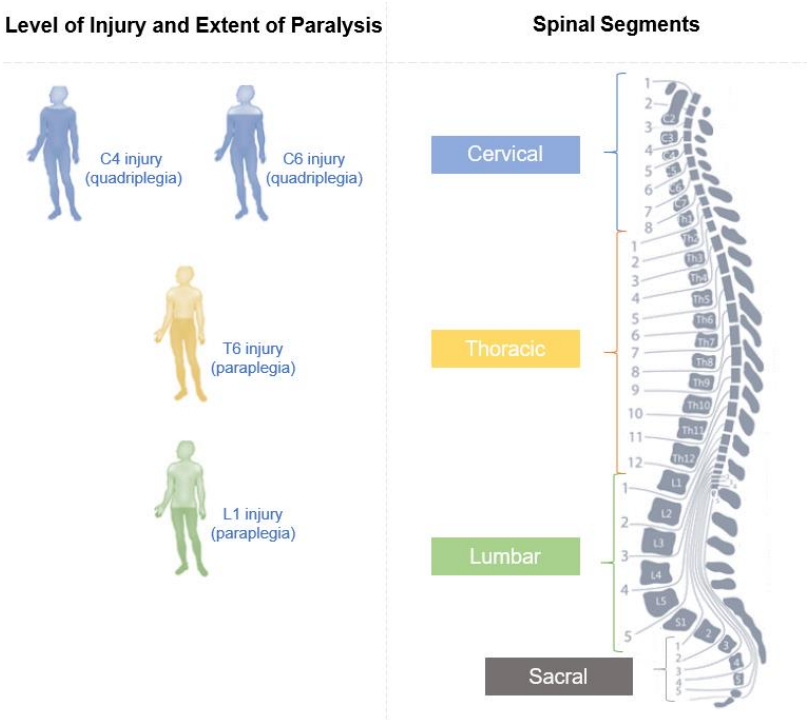


Figure 16 Level of lesion and extension of paralysis according to the spinal segment.

Myelopathy is a late complication and many patients may not have had enough time to manifest it. The risk of radiation myelopathy from spine SBRT has been estimated to be <1% [43].

RM is a rare late toxicity effect in the modern era of 3-dimensional conformal conventionally fractionated RT. This devastating late effect has re-emerged as a direct result of SBRT practice, where high-dose radiation is delivered adjacent to the spinal cord to be spared. Possible reasons include [57]:

I. Lack of understanding of tolerance to the spinal cord with high dose per fraction radiation therapy;
II. Sensitivity of the spinal cord in SBRT dose distribution to small intrafraction motions where even 1- to 2mm shifts during treatment can increase the actual spinal cord dose delivered;
III. An assumption that a small point volume within the spinal cord can tolerate a higher dose of radiation than would traditionally be considered safe.

Nieder *et al*. [31], developed a criteria for select patients at risk of developing myelopathy from 3 criteria: Cumulative BED in Gy_2, interval between first treatment and re-treatment is < 6 months and BED value in the first treatment is >102Gy_2.

From these data we can group the patients into three different groups: low-risk, intermediate-risk and high-risk groups. If the patient gets less than 3 points belongs to the low risk group, if he has 4 to 6 points belongs to the intermediate risk group, and finally if he has more than 6 points belongs to the group of high risk of developing myelopathy (Table 12).

Table 12 Risk of developing myelopathy from the characteristics mentioned in the table. Adapted from [31].

Points	Cumulative BED in Gy_2 with interval < 6 months and a BED of 1 course ≥102 Gy_2	Risk of developing myelopathy
0	≤ 120.0	Low risk
1	120.1 – 130.0	
2	130.1 – 140.0	
3	140.1 – 150.0	
4	150.1 – 160.0 x (4.5) x (4.5)	Intermediate risk
5	160.1 – 170.0	
6	170.1 – 180.0	
7	180.1 – 190.0	High risk
8	190.1 – 200.0	
9	> 200.0	

A bibliographic search was conducted in *PubMed*, *Medline*, among others, to identify articles with references of SBRT in cases of re-irradiation and the effect of different dose/fractionation regimes. Table 13 presents information regarding 1st treatment and re-irradiation data from several studies. Also included are values regarding:

- Total dose and the number of fractions delivered, both in the initial treatment and in the re-treatment;
- Time elapsed between the end of the first treatment and the re-treatment;
- α/β ratio;
- Treatment area (cervical, thoracic, lumbar and sacral);
- Follow-up time;
- Time until radiation myelopathy appears, should it occur.

Based on the classification developed by Nieder *et al*., (Table 13): when the interval between the two treatment courses is not shorter than 6 months and the dose of each course is BED ≤98Gy_2, the cumulative BED where no case of radiation myelopathy (RM) has been reported is 120Gy_2; for values between 120 and 135 Gy_2, the risk of RM is small [31].

Analyzing table 13 it is possible to highlight the following:

- Data presented by Wong *et al*. [71]: patient received 20.6Gy in the 1st treatment in 6 fractions and was retreated after 4 months.
  BED for 2nd treatment was 102.65Gy_2; cumulative BED was 158.58Gy_2. His risk score was 4, which presented an intermediate risk of developing myelopathy, which occurred after 6 months;
- Data presented by Schiff *et al*. [72], Grosu *et al*. [67], Nieder *et al*. [31] and Maranzano *et al*. [73]: the cases reported had a time interval between treatments of more than 6 months, BED is ≤ 98Gy_2 for each treatment, cumulative BED = 120Gy_2 and no cases of myelopathy;
- Data presented by Sahgal *et al*. [40]: patient had a time interval between treatments of 5 months; cumulative BED= 204.4Gy_2 and a high risk of developing myelopathy (score of 9). The follow-up time was very short (4 months) for the manifestation of symptoms, should they occur;
- Data presented by Sahgal *et al*. [57]: 9 cases of patients developing myelopathy were reported. BED values ranged from 181.3 to 337.5 for the re-treatment. Time to manifestation of symptoms in this group of patients ranged from 3 to 15 months.

Table 13 Summary of published reports of treatments performed using re-irradiation.

		1st treatment			Re-irradiation								
Author	Sample	Total dose (Gy)	Dose fx (Gy)/no fx	Interval (months)	Total dose (Gy)	Dose fx (Gy)/no fx	Nivel treated	Value of α/β (Gy)	Initial BED	Irradiation BED	Total BED	Follow-up (months)	Time to myelopathy (months)
Wong *et al*., 1994 [71]	11	2.0+45.9	2.0/1+3.06/15	12	23.6	2.36/10	T	2	120.13	51.45	171.58	≥60	4
		23.5+18.2	2.35/10+3.64/5	19	13.6	2.72/5	T		102.44	32.096	134.54		16
		20.6	3.43/6	4	36.4	3.64/10	T		55.93	102.65	158.58		6
		20.1	1.26/16	48	14.3+3.2 +22.6+2.57	2.38/6+1.6/2+ 3.76/6+2.57/1	T		33.25	108.04	141.29		11
		16.0	3.2/5	57	22.0	7.33/3	T		41.60	102.63	144.23		7
		24.0+24.0	3.0/8+1.5/16	45	18.1	1.81/10	T		102.00	34.48	136.48		13
		20.4+20.3	4.08/5+4.06/5	10	8.2	8.2/1	T		123.53	41.82	165.35		4
		24.4	4.88/5	2	17.0	3.4/5	T		83.94	45.9	129.84		14
		31.2	1.56/20	71	30.3	5.05/6	C		55.54	106.81	162.35		25
		33.9	3.39/10	2	25.6	2.56/10	T		91.36	58.9	150.4		13
		50.0	2.5/20	27	10.4	1.04/10	T		112.50	15.81	128.31		11
Schiff *et al*.,1995 [72]	54	30.0	3.0/10	Median 9.1 (range: 1-51.3)	30.6	1.8/17	T	3	60.0	48.96	108.96	4.2	No cases
		30.0	3.3/9		39.6	1.8/22	L		63.0	63.36	126.36		
		30.0	3.0/10		22.0	2.0/11	C/T		60.0	36.7	96.7		
Sminia *et al*., 2002 [56]	8	16.0	8.0/2	52	20.0	4.0/5	T	2	80.0	60.0	140.0	1-54	No cases
		39.0	3.0/13	12	21.0	3.0/7	T		97.5	52.2	149.7		
		8.0	8.0/1	4	18.0	3.0/6	C		40.0	45.0	125.0		
		49.6	1.6/31	61	50.0	2.0/25	L		69.4	75.0	144.4		
		37.4	2.2/17	30	21.0	3.0/7	T		78.5	52.2	130.7		
		24.0	3.0/8	20	21.0	3.0/7	T		60.0	52.2	164.4		
		25.0	1.7/15	150	16.0	4.0/4	T		46.0	48.0	142.0		
		35.25	1.5/23.5	73	30.0	3.0/10	L		48.5	52.5	125.0		
Grosu *et al*., 2002 [67]	8	40.0	1.25/32	Median 30 (range: 6-63)	29.0	1.8/16	T	2	65.0	55.1	120.1	Median 16 (range: 5-44)	No cases
		30.0	3.0/10		30.0	2.0/15	L		75.0	60.0	135.0		
		40.0	2.5/16		30.6	1.8/17	T/L		90.0	58.1	148.1		
		40.0	2.0/20		30.6	1.8/17	T		80.0	58.1	138.1		
		50.0	2.0/25		18.0	3.0/6	T		100.0	45.0	145.0		
		30.0	3.0/10		30.0	2.0/15	T		75.0	60.0	135.0		
		29.0	1.4/20		30.0	3.0/10	T		49.4	75.0	124.4		
		36.0	3.0/12		30.0	2.0/15	L		90.0	60.0	150.0		
Wright. *et al*., 2006 [68]	37	40.0	2.67/15	Median 19 (2-125)	20.0	4.0/5	SC	2	93.0	11.0	104.0	8 (range 1-51)	No cases
		30.0	3.0/10		20.0	4.0/5	CE	4	53.0	4.0	57.0		
		30.0	3.0/10		20.0	4.0/5	SC	2	75.0	17.0	92.0		
		50.4	1.8/28		20.0	4.0/5	CE	4	73.0	9.0	82.0		
		37.7	2.51/15		20.0	4.0/5	SC	2	85.0	20.0	105.0		

T, thoracic; C, cervical; L, lumbar; SC, spinal cord; CE, cauda equina. BED is calculated using the formula: Eq.3.

Table 13 (cont.) Summary of published reports of treatments performed using re-irradiation.

		1st treatment			Re-irradiation								
Author	Sample	Total dose (Gy)	Dose fx(Gy)/no fx	Interval (months)	Total dose (Gy)	Dose fx(Gy)/no fx	Nivel treated	Value of α/β (Gy)	Initial BED	Irradiation BED	Total BED	Follow-up (months)	Time to myelopathy (months)
Nieder *et al*., 2006 [31]	7	60.0	2.0/30	37	64.0	2.0/32	C-T	2	37.5	70.0	107.5	7	No cases
		60.0	2.0/30	31	56.0	2.0/28	C	2	37.5	67.0	104.5	5	
		70.0	15 x 2 Gy followed by twice-daily radiotherapy with 1.8 Gy in the morning and 1.5 Gy in the afternoon	23	50.0+12.0	2.0/25+3.0/4	C	2	80.0	43.0	123.0	10	
		59.4	1.8/33	12	45.0	1.8/25	C	2	96.0	85.5	181.5	8	
		36.0	3.0/12	30	30.0	2.0/15	L-S	4	63.0	39.0	102.0	20	
		44.8	1.6/10+1.8/16	96	36.0	2.0/18	T	2	83.5	72.0	155.5	5	
		40.0	2.5/16	26	30.0	2.0/15	L	4	65.0	45.0	110.0	17	
Maranzano *et al*., 2011 [73]	12	8.0	8.0/1	31	8.0	8.0/1	SC	2	40	40.0	80.0	SI	No cases
		8.0	8.0/1	9	16.0	2.0/8			40	80.0	120.0		
		8.0	8.0/1	9	8.0	8.0/1			40	40.0	80.0		
		8.0	8.0/1	5	8.0	8.0/1			40	40.0	80.0		
		8.0	8.0/1	2	8.0	8.0/1			40	40.0	80.0		
		8.0	8.0/1	4	20.0	5.0/4			40	60.0	120.0		
Sahgal *et al*., 2012a [40]	19	37.5	2.5/15	5	24.0	8.0/3	C	2	84.4	120	204.4	4	No cases
		39.1	2.3/17	39	25.0	5.0/5	L		84.1	87.5	171.6	7	
		30.0	3.0/10	6	24.0	6.0/4	L		75	96	171	5	
		50.0	2.0/25	11	24.0	8.0/3	T		100	120	220	8	
		40.0	1.82/22	81	20.0	10.0/2	T		76.4	120	196.4	55	6
		25.2	0.9/28	70	21.0	10.5/2	T		36.5	131.3	167.8	29	5
		21.2	4.24/5	11	14.0	14.0/1	T		66.1	112	178.1	17	3
		51.9	1.85/28	18	33.0	11.0/3	C		99.9	214.5	314.4	11	8
		43.2	2.88/15	12	16.0	16.0/1	T		105.4	144	249.4	3	3
Sahgal *et al*., 2012b [57]	66				25.0	12.5/2	T			181.3		17	9
					24.0	8.0/3	C-T			120		9	9
					16.0	16.0/1	Clivus-C1			144		24	13
			NR		25.0	25.0/1	C	NR		337.5	NR	40	13
					25.0	25.0/1	C			337.5		39	5
					18.0	18.0/1	T			180		24	12
					16.0	16.0/1	C			144		8	3
					14.0	14.0/1	T			112		16	6
					30.0+14.0	3.0/10+14.0/1	C			187		23	15

T, thoracic; C, cervical; L, lumbar; S, sacral; SC, spinal cord; NR, not reported. BED is calculated using the formula: Eq.3.

4.2.2 Pathophisiology

Radiation injury to the spinal cord is among the most studied experimental models of radiation injury and probably is the most fully documented clinical radiation complication.

To the thoracic cord, it is still apparent that its sensitivity to radiation is substantially less than that of the cervical cord. A significant oxygen effect exists, and it is possible that the thoracic cord is intrinsically less well oxygenated. Extrinsic conditions, such as smoking history or effects of lung cancer may result in the patients with thoracic irradiation having less well oxygenated nervous tissues. Haemoglobin levels are significantly greater in thoracic patients with myelopathy that in those without myelopathy treated to the same dose [74].

The incidence of cervical myelopathy at 45Gy is, approximately, 0.03% and at 50Gy is, approximately 0.2%. Large lengths of cord, concomitant chemotherapy and other factors may increase this incidence [74].

Spinal cord, besides being one of the most dose-limiting organs in radiotherapy, is also a risk organ present in several neoplasms. It is a serial architectural organ, being very sensitive to radiation in small portions of its extension. There is a possibility that the sensitivity of the spinal cord depends on the organization and structural distribution of its functional subunits (FSU), since there are structural differences between the different spinal segments. These differences are mainly patented in grey and white matter [51, 52].

Histolopathology, changes of late lesions may include [46]:

- Reactive gliosis;
- Demyelination;
- Necrosis confined to white matter;
- Changes in both white and grey matter.

In rat studies it was found that after delivery of single or fractionated doses in the spinal cord, rats developed paralysis of the lower limb 4 to 7 months after necrosis of the white matter. Necrosis and demyelination of the white matter occurs generally in the absence of gross vascular abnormalities. Functional deficits associated with vascular damage are variable, and they are observed much later and after lower doses in rodents and in human myelopathy [46].

Recently, animal studies have been conducted on the influence of small volumes and non-homogeneous irradiation with steep dose gradients along the cord to simulate

SBRT. The data obtained were conflicting. In the case of the rat, it was observed that when irradiating the spinal cord there was a significant volume effect after proton irradiation and that there was evidence of differential regional radiosensitivity in the spinal cord. On the other hand, in studies on pigs it was found that no significant volume dependence was observed. These cord irradiation studies described histopathological changes like the studies using homogeneous doses along the cord [46].

One of the characteristics of delayed spinal cord injury is demyelination. Oligodendrocytes are the cells responsible for myelination in the lesion response. After irradiation, oligodendrocytes and oligodendrocyte progenitor cells (OPCs) were observed to undergo apoptosis in the rodent spinal cord due to a p53-mediated response. The assay demonstrated an initial loss of the clonogenic OPC pool in rat spinal cord after irradiation. Followed by dose dependent recovery and a second decline between 4 and 5 months after paralytic doses. So far, there is no evidence of a causative association between acute oligodendroglial apoptosis after irradiation and late observed demyelination.

The fundamental biologic mechanism of RM remains unclear. The actual model suggests that mitotic death of endothelial cells results in blood-spinal cord barrier (BSCB) disruption. This disruption leads to vasogenic edema, hypoxia and an inflammatory cascade resulting in demyelination and necrosis.

The effects arising from the volume of irradiated bone marrow may be related to vascular supply, collateral circulation and/or the ability to re-establish damaged vasculature through revascularization. There is also the issue of the release of cytokines and mediators of inflammation. Their release may be affected by the irradiated volume because the larger the volume the greater the release of these potentially harmful substances [45].

In summary, recovery from initial damage is well established. The amount of recovery seems to be influenced by different factors, with initial damage expressed as a percentage of cord tolerance being the most important one. Time interval between the first treatment and re-treatment is also important as it is estimated as percentage recovery.

The real total tolerance of the spinal cord may be about 130% of the tolerance dose for the first treatment as estimated by Kogel [32]. Although the general phenomenon of repair occurs in humans, caution is necessary when translating experimental animal data to the clinical setting because of the life span, latency and dose response difference [75]:

- Histological pattern of myelopathy in animals differs from those found in humans;
- Repair kinetics is probably different, and the time to achieve maximal repair and re-tolerance in humans [76]. It is of extreme interest to consider the knowledge about the kinetics of the evolution of the hidden damages caused by the previous treatment.

In general, a higher re-treatment dose can be given following lower initial doses and longer intervals between treatments. From the sparse clinical and primate data, it appears that at least 50% recovery from 45Gy would be obtained 2 years after treatment.

4.2.3 Dose selection

Initially, radiation oncologists using SBRT used ablative hypofractionation, i.e., few fractions with a dose greater than 8Gy per fraction, to create a more drastic impact on cancer treatment [66].

Several studies were carried out, where the only variable was the radiation dose. In these studies, 25 to 100 patients were registered, which allowed an assessment of toxicity and early efficacy. This phase of treatment allowed the discovery of the most effective dose, which defines the limits of treatment in a shorter period, since patients were exposed to very low or very high doses [66].

The choice of patients is a very important step, especially when it comes to a re-treatment. Decisions must consider the current situation of the patient: the degree of systemic disease, the time between the first and second irradiation, the time of local relapse, and if there was surgery (Table 14) [77].

The strengths of SBRT treatment include high rates of tumor eradication through convenient and non-invasive outpatient treatment. On the other hand, the most observed disadvantages are the possibility of causing toxicity, such as ulceration, stenosis, fibrosis and even necrosis, which can occur considerably later after treatment [66].

Patients undergoing SBRT treatment should have a stable spine that is assessed through the Spinal Instability Neoplastic Score (SINS). SINS stratify the risk of lesions by location, pain, type of injury, alignment of the spine and the presence of collapse of the vertebral body and posterolateral involvement of spinal levels [60].

Table 14 The inclusion and exclusion criteria for SBRT. Adapted from: [47, 60, 63].

General criteria	
Inclusion	Exclusion
Good performance status	Moderate and poor performance status
Oligometastatic disease in ≤5 sites extracranial metastases	Oligoprogression in patients with metastatic and/or rapidly progressive disease with limited life expectancy
Oligoprogression in patients with oligometastatic disease	Bone compression of neural structures; can cause spinal instability or neurological deficit
Without or with minimal spine instability	With spine instability
Without or minimal epidural disease, i.e., Bilsky 0-1	With moderate or high grade of epiural disease, i.e., Blisky 2 or 3
Tumor is involved no more than 3 spinal levels (contiguous or non-contiguous)	More than 3 spinal levels involved and/or presence of diffuse spinal disease
Histology of the tumor called “radioresistant”	Histology of the tumor called “radiosensitive”
Failure to treat conventional radiotherapy previously performed	Delivery EBRT within less than 5 months before performing SBRT treatment
First treatment of SBRT delivered within 5 months or more before a second treatment of SBRT	First treatment of SBRT delivered within 3 months or less before a second treatment of SBRT
Detection of spinal metastases through CT and MRI	Incapability to support a near-rigid or supine immobilization due mechanical spine instability
High risk of recurrence or gross residual disease	Systemic disease out of control
Gross tumor more than 2 mm from spinal cord without signal for initial surgery	Presence of significant or progressive neurological deficit
Impossibility of surgery due to the existence of two or more diseases simultaneously in the same patient or due to the refusal of the patient	With connective tissue disease.
Histological confirmation of neoplastic cells in the patient	Limited survival

The best survival rate has been verified with female patients, with better status performance, based on previous surgery at the SBRT site, presence of solitary spinal metastasis, and a long disease-free interval [60].

A multi-institutional study demonstrated how re-treatment using SBRT is safe and effective. The initial treatment, performed with conventional radiotherapy, was prescribed with doses of 3Gy per fraction, completing a total of 10 fractions. In the re-treatment, mean doses of SBRT were 16.6Gy in 1 fraction or 24Gy in 3 fractions. After 6 and 12 months of re-treatment, local control was 93% and 83%, respectively. The reported toxicity was vertebral compression fracture (VCF), with a rate of 4.5%, and there were no reports of cases of myelopathy due to radiation [60].

According to *Huo et al.* [60], re-treatment using single fraction SBRT was able to predict better local control since it showed local control rates in a period of more than one year between 66% and 93%. On the other hand, studies using lower prescription doses, i.e., 20Gy in 2 fractions and 20Gy in 5 fractions, showed lower rates of local control.

Fractionation differentiates the intrinsic radiosensitivity between the tumor and the spinal cord, allowing high doses of radiation to be safely delivered to lesions within the epidural space, where up to half of all recurrences occur after conventional radiotherapy.

4.2.4 Vascular damage in tumors

Initially, clinical trials were performed in the liver and lung for the treatment of primary and metastatic liver and lung tumors. These organs have parallel hierarchy, which leads to a lower tissue toxicity when irradiated.

SBRT was tested in patients whose lung tumors were inoperable and had relatively small targets, making dosimetry more reasonable in the normal lung. As results, the control rate in primary tumors with SBRT was found to be within the range of 80%. However, the toxicity rates were quite low, with values between 15% -20%, even in the most fragile patients [66].

In a 1g tumor, it is known that there are up to 10^8-10^9 cells, suggesting that 8 to 9 log tumor cells should be eradicated to control tumors with this mass. The calculation by Brown *et al*., using conventional radiobiological principles, indicated that the radiation doses used in SBRT are insufficient to kill all clonogenic cells in tumors with a diameter of 1-3cm, if 10-20% of tumor cells hypoxic. On the other hand, SBRT was effective in the treatment of tumors with diameters greater than 5-6 cm when irradiated with 30-60 Gy in 2 to 5 fractions. Thus, SBRT, in addition to causing direct cell death, also causes indirect cell death through vascular damage, which occurs when the tumor is exposed to high-dose hypofractionated radiation [62].

New tumor vessels form due to the increasing need for nutrients, including oxygen in growing tumors. These blood vessels are composed of a single layer of endothelial cells, incomplete and disorganized basement membranes. Tumor endothelial cells, which are irregularly shaped, are linked to others with large gaps between them, which are obstructed by tumor cells [62].

Abnormal blood vessels in the tumor have gaps between endothelial cells or incomplete basement membrane support. Although there is little information on the effects of high dose hypofractionation on human tumor vasculatures, there are many reports on the effects of high dose hypofraction on the vasculature of experimental tumors.

Park *et al*., reported that endothelial cells retired from human breast cancer were more radiosensitive when compared to endothelial cells from adjacent normal breast tissues.

Although human functional vasculature remains unchanged or better during the initial period of conventional fractional radiotherapy, it gradually declines during the latter part of the treatment. Irradiation at doses greater than 10Gy in a single fraction or 20-60Gy in a limited number of fractions causes severe vascular damage leading to indirect death of tumor cells due to the acute decrease in blood perfusion, making the tumor environment hypoxic and without nutrients [58, 62].

4.2.5 Tumor hypoxia and SBRT

Tumor hypoxia occurs in most human solid tumors (Figure 17). This feature contributes to the failure of many anticancer therapies such as surgery, chemotherapy and radiotherapy [78]. Hypoxia occurs due to uncontrolled proliferation of cancer cells once the tumor exhausts the nutrient and oxygen concentration from the normal vasculature and becomes hypoxic.

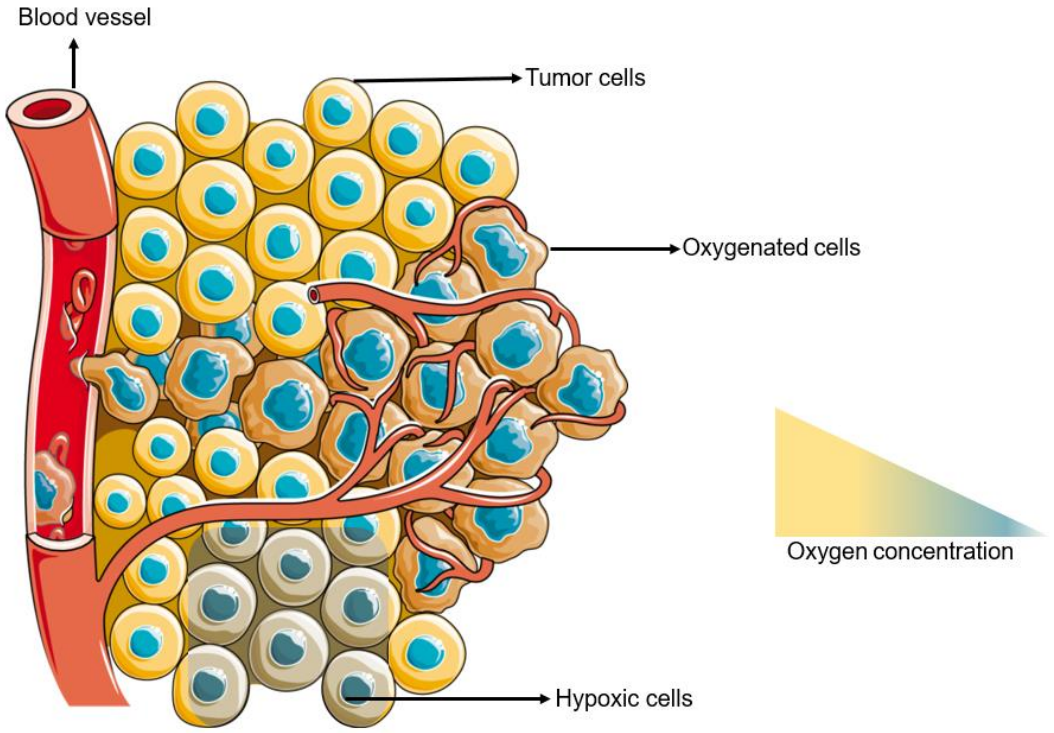


Figure 17 Hypoxic tumor. Near the blood vessel the tumor has a lot of oxygen but the greater the distance the cells to the blood vessel the lower the oxygen concentration.

Recent modelling by Carlson *et al*., suggested that assuming daily fractionation and full reoxygenation between fractions, tumor hypoxia is actually more serious problem with SBRT (Figure 18) [78].

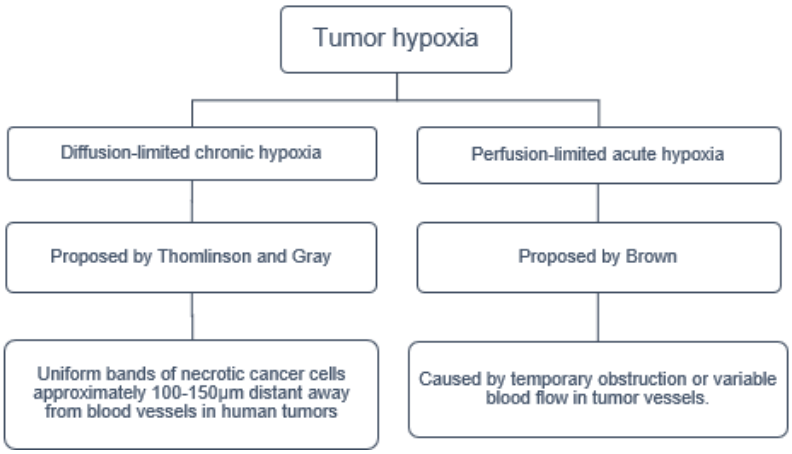


Figure 18 Tumor hypoxia can occur through two different mechanisms proposed by Thomlinson and Gray and Brown. Adapted from [78].

The following graph, adapted by Carlson *et al*., shows that the survival of tumor cells can increase up to 100 times when we apply a smaller number of fractions and a higher dose per fraction, which is observed by the letter A. This can represent a situation equivalent to a SBRT treatment. In letter B, an increase in hypoxic fraction from 10% to 30% reflects nearly 10 times further resistance of tumor cells at this high dose hypofractionated regimen in this modelling study [78].

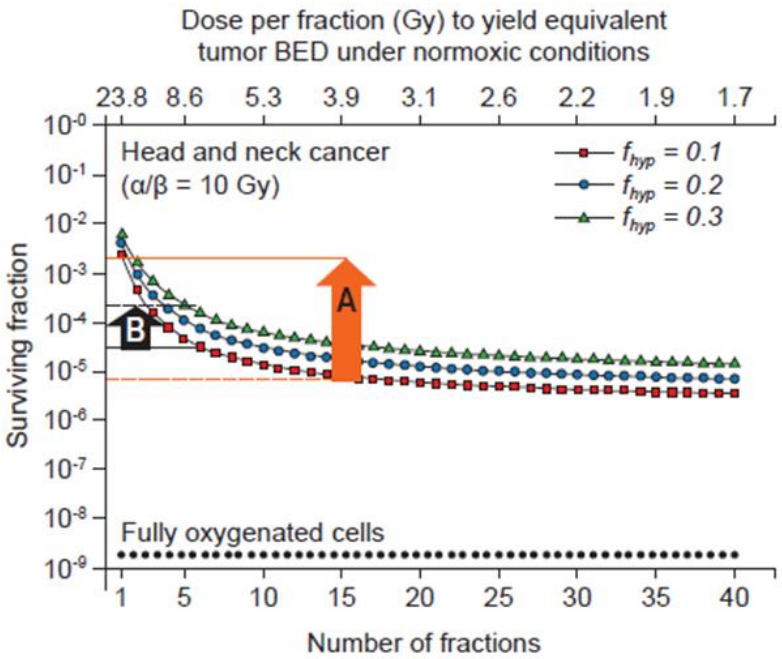


Figure 19 Survival curve of tumor cells as a function of dose per fraction supplied. It is assumed that daily fractionation and complete reoxygenation occurred between fractions. Adapted from [78].

Description of the letters shown in figure 19 [78]:

- **A**: For tumors with 10% hypoxia (f_{hyp} = 0.1; red line) there is more than 2 log (100-fold) of cell survival advantage (pointed with the orange arrow) if reduce the number of fractionations (as we shift right to left);
- **B**: As hypoxic fraction increases from 10% (f_{hyp} = 0.1; red line) to 30% (f_{hyp} = 0.3; green line), there is additional 1 log (10-fold) of cell survival increase (pointed with the black arrow) at 5 fractionation treatments, at which the cell survival is already being much higher than that by hyperfractionation.

One problem of SBRT is that there is no standard interval between each hypofraction: Several studies were performed with different fractionation intervals and all presented good clinical results. Some of the plans described by different authors were: Timmerman *et al*. [66], prescribed 18Gy per fraction × 3 fractions within 2 weeks, while Nagata *et al*. prescribed 12Gy per fraction × 4 over 5–13 days. In one study 50Gy was delivered over 4 consecutive days, meaning 12.5Gy every day for 4 days [79].

To determine changes in tumor hypoxia before and after a single fraction of 20Gy, Hong *et al*. [78], used various techniques such as F-MISO PET imaging, pimonidazole and bioluminescence imaging in a mouse tumor model. It was possible to conclude that tumor hypoxia did not change significantly after irradiation. However, it was found that after high dose irradiation, the cells had a transient vascular collapse.

Radiosensitizers are combined with the radiation to render tumor cells more sensitive when irradiated (Table 15).

Table 15 Example, nimorazole has better results at high doses (doses being equivalent to dose used in SBRT) [78].

Combination of treatment	Value of TCD_{50} (Gy)
Radiation: 3 fractions x 15Gy	29.7
Radiation + 1 x nimorazole	20.1
Radiation + 3 x nimorazole	2.5

Wittenborn and Horsman have demonstrated that the dose of radiation to control 50% of tumors (TCD_{50}) can be reduced from 29.7Gy to 2.5Gy when 3x15Gy was combined with nimorazole 30 minutes prior to each fraction [78].

4.2.6 Rs impact

The classical fractionation principles, *i.e.*, the Rs of radiobiology, explain the effects of high doses of ionizing radiation on tumors and adjacent normal tissues. Like conventional fractional radiotherapy, it is of interest to refer to how these biological mechanisms apply in this new method of radiotherapy (Table 16).

Some radiobiological questions remain without response regarding the evaluation of the doses administered in the SBRT and the effect of high doses per fraction. While in conventional radiotherapy the treatment time is approximately 5 minutes, in the SBRT the treatment session takes about 15-40 minutes

Table 16 Impact of radiobiological mechanisms in SBRT treatment.

Biological mechanisms	Description
Repair of sublethal damage	Treatment with SBRT has a longer irradiation time when compared to conventional radiotherapy. With this treatment, repair of sub-lethal damage may occur during prolonged exposure to radiation. After 30 minutes, the sublethal damage repair is greater than >10% [62, 80].
Repopulation of cells after radiation	Repopulation occurs depending on the type of tissue and the dose of radiation administered. In conventional radiotherapy, tumor cell repopulation occurs within 2 to 3 weeks after start of RT. Thus, it is possible that the repopulation of tumor cells may occur earlier in the treatment of SBRT or SRS than in conventional RT [62]. Delivering a higher dose should mitigate any clonal expansion and thus offer a significant advantage to rapidly dividing tumors [76]. As treatment time of SBRT lasts is short, 2 weeks at most, repopulation of tumor cells will not be a problem [80].
Redistribution of cells within the cell cycle	Reducing the number of fractions alters the probability of irradiating a cohort of cells as they move into a radiosensitive phase [81]. After irradiation with a single fraction of 15–20Gy, cells are indefinitely arrested in the phases of cell cycle where they were irradiated and undergo interphase death. After irradiating the cell cycle with a dose of 20Gy in a single exposure, cell cycle progression was delayed, and many cells underwent necrosis in the phases of the cycle where they were irradiated. The cells that were irradiated slowly progressed to the G2 phase and died [62].
Reoxygenation of the surviving cells	In SBRT, due to the vascular damage caused by the high doses of radiation, the intratumoral environment becomes hypoxic. Hypoxic cells reoxygenated when irradiated with a relatively low dose per fraction, i.e., less than 10Gy [80]. Following administration of a SBRT or SRS treatment, massive vascular destruction of the tumor occurs, and reoxygenation of hypoxic cells occurs 2-3 days after irradiation. As oxygen consumption declined dramatically after massive death of tumor cells, surviving hypoxic cells can be reoxygenated. Changes in the oxygenation status of the tumors after irradiation with high doses are still unclear [62]. SBRT has a theoretical disadvantage because of the short delivery time. Although this might be compensated by the high doses per fraction.
Radiosensitivity	SBRT attenuates differences in tumor death that are directly attributable to variations in radiation sensitivity of individual tumor cells. Large doses per fraction and short treatment time used in SBRT provides less opportunity for the development of resistant stem cells [81].

Regarding radiobiology, it is of interest to mention how these biological mechanisms apply in this method of radiotherapy. The major question raised is whether the dose of radiation administered in intervals is equivalent to that administered without breaks, since repair of sublethal damage occurs within the time interval between the administration of two doses of radiation [62, 79]. According to Kim *et al*. [62], it is indicated that radiobiological factors are not relevant to the single dose in SBRT or SRS.

Tumor hypoxia may actually be a more serious problem with SBRT due to a reduction in reoxygenation between fractions as associated with small number of SBRT fractions (1 to 5) in comparison with conventional RT. This problem could be overcome if the next fraction of SBRT could be to deliver after tumor reoxygenation. And although there are no standard intervals between the high doses of SBRT, good clinical results were obtained despite different fractionation intervals [78].

As SBRT uses doses per fraction much higher than conventional radiotherapy, the radiobiological mechanisms underlying radiation response have been subject to controversy, regarding their application to extreme hypofractionation [79]. Hypofractionation does not follow the basic principles of radiobiology to explain tumor responses obtained by conventional fractionation schemes.

Thus, the relevance of the Rs of radiobiology in a scenario of hypofractionation is controversial: As the treatment time decreases, repopulation will be of lesser importance and the clinical significance of cell redistribution in the cell cycle, in a scenario of hypofractionation, is unclear. The advantages and disadvantages of the radiobiological mechanisms are presented in (Table 17).

In spite of the controversy around the relevance of the 5Rs in SBRT, [62, 82], it is now clear that these radiobiological concepts are sufficient to explain the impressive results obtained from clinical studies are the result of much larger biologically effective doses delivered [83].

Table 17 Advantages and disadvantages of radiobiological mechanisms. Adapted from [81].

Radiobiological mechanisms	Advantage	Disadvantage
Repair	Improvements in the technique of dose delivery allows dose reduction delivered to adjacent normal tissues.	Limitation of the number of cycles of damage and repair that separate the tumor response from normal tissue toxicity.
Repopulation	Tumor repopulation is greatly reduced or eliminated during a treatment with less fractions. It is specifically relevant for radiation-resistant tumor stem cells.	None
Redistribution	The distribution of the cells will be affected by the reduced number of fractions. The clinical significance is still unknown, so it is not possible to enumerate the advantages and disadvantages of this biological mechanism.	
Reoxygenation	None	A smaller number of fractions potentially reduces reoxygenation between fractions, which results in an increase in radioresistance.
Radiosensitivity	A single fraction dose greater than 10Gy may trigger apoptosis of endothelial cells.	Normal late-reacting tissues are more radiosensitive at higher doses per fraction and so the risk of late complications is higher at higher doses per fraction.

4.2.7 Linear-Quadratic Model

The linear-quadratic (LQ) model is advantageous for the calculation of iso-effect doses in the treatment of cancer with conventional multi fractionated radiotherapy. This model assumes that two-strand DNA breakdown is responsible for radiation-induced clonogenic cell death and that hypoxic cells are fully reoxygenated during the fractional irradiation interval [62].

Due to the quadratic component in the formula, many authors have suggested that the LQ model is used to overestimate cell death with increasing radiation dose. Interestingly, despite the problem inherent in the LQ model, some researchers have reported that the LQ model fits certain clinical results of SBRT and stated that direct cell death due to DNA damage is enough to explain the high clinical efficacy of SBRT. The tumor cell survival curve in vivo curves downward as the radiation dose increases above approximately 10Gy, which occurs due to secondary cell death caused by vascular damage [58, 62].

Thus, it is permissible that, in a given clinical situation, cell death calculated by the LQ formula incidentally does not exceed estimates, but approximates total SBRT cell death, which encompasses not only direct and indirect cell death [62].

4.2.8 Limitations and constraints of SBRT

The dose limitations for the spinal cord in an SBRT treatment are [46]:

- Limiting the spinal cord point maximum volume to a safe dose, based on a conventional fractionation, of 8 to 10Gy;
- Limiting the safe dose to a larger volume and / or volume of the irradiated cord, thus the maximum dose within the cord is higher than that which was thought to be tolerable;
- Limit the safe dose of the spinal cord to the thecal sac or to the conjugation of the spinal cord with a margin of uncertainty of 1.5 mm beyond the cord and not the "true" cord itself to reduce the technical uncertainties in dose delivery.

Brenner *et al*. (2008) [33] described the use of the LQ model applied to doses within the range of extreme hypofractionation.

In vitro and *in vivo* experiments demonstrated that:

- *In vitro* experiment: Garcia *et al.*, conducted a research where they used a colony test to measure cell survival. The surviving cell fractions were recorded in the 0-16Gy dose region. To estimate the dose regions where the single-fraction LQ model adjusted the data, the authors of this study adjusted the data from 0-4Gy and then 0Gy to progressively higher doses, verifying if the model fit the bottom of the dose increase. In summary, it was verified that the LQ model does not decrease its fit quality until doses above 15Gy [33].
- *In vivo* experiment: There were several quantitative parameters in vivo that allow to verify the agreement of the LQ model in the 2-20Gy range. The shape of the graph, presented in figure 20, called “the reciprocal dose Fe graph”, is such that if the LQ model applies the inserted data would form a straight line. The portion of the reciprocal dose provides a visual indication of how in vivo data matches the LQ model in the dose range of interest. The quantitative parameters depicted in the following image are consistent with the LQ model, over a wide range of doses per fraction, including doses of interest in hypofractionation [33].

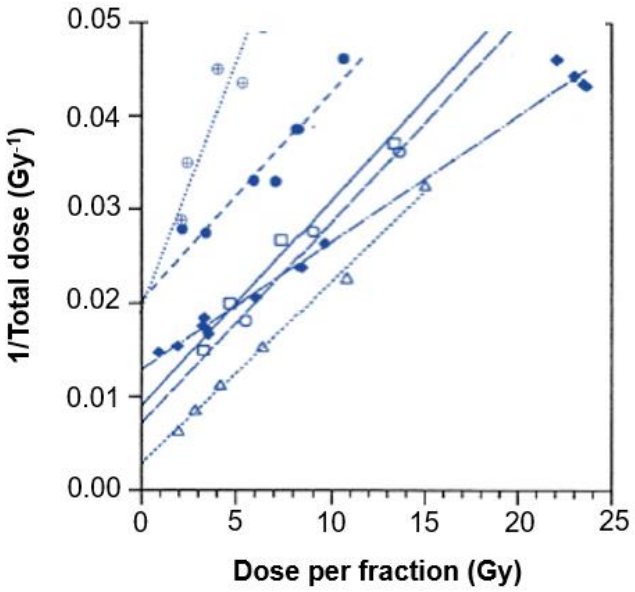


Figure 20 Iso-effect data for late response from 3 different regions, represented by □○▲, of the rat spinal cord. Where ◊ represents acute skin reactions in mice, ● for early and ⊕ late murine intestinal damage. The data are plotted in a "reciprocal-dose Fe" form26 such that, if they follow an LQ relationship, the points fall on a straight line. Adapted from [33].

Brenner *et al*., [33] concluded that the LQ model is mechanically plausible for designing protocols in the dose range by fraction of 2-10Gy, above 10Gy and the model is expected to become progressively less accurate. However, animal studies have shown that the model is acceptable in clinical trials with 15-18Gy fractions. There are more sophisticated methods for assessing agreement with the LQ model, due to the uncertainties inherent in the data. However, with the studies analysed, the LQ model applies to fractions of 20Gy, approximately.

As radiosurgery is increasingly being employed in the treatment of spinal lesions, and although reports of toxicity are rare, the follow-up time is short and patient numbers small. The effect of concurrent chemotherapy is essentially unknown in that situation.

Also, the uncertainty in dose calculation results in BED uncertainty and the difference between the prescribed dose and the dose given are limiting factors of the data published so far [31].

Most studies related to re-irradiation are based on data from retrospective studies or small prospective studies. In addition to this, re-irradiated populations are more heterogeneous than patients with local or regional recurrence or second tumors [6].

The major paradigm associated with spinal cord re-irradiation is related to the lack of evidence regarding repair of long-term damage in this organ. There is a greater uncertainty related to tolerance rates during re-irradiation, since most of the existing data are derived from results obtained with animals, revealing long-term damage recovery [5].

As SBRT is increasingly being employed in the treatment of spinal lesions, and although reports of toxicity are rare, the follow-up time is short and patient numbers are still small. The effect of concurrent chemotherapy is essentially unknown and needs to be investigated.

The consensus is that SBRT is appropriate in the postoperative cases in patients with limited disease, radioresistant tumors and as another treatment strategy after failure of conventional radiotherapy treatment.

The optimal dose prescription for SBRT remains controversial. On the one hand, a single fraction treatment results in better local control rates than obtained with conventional RT. This may be the result of larger biologically effective doses delivered with SBRT. Alternatively, higher rates of local control following single fraction treatment in retrospective series may simply reflect better outcomes in patients with less aggressive disease [84].

4.2.9 Consensus guidelines

15 radiation oncologists and 5 neurosurgeons, representing 19 centers in 4 countries and having experience in 1300 cases of spine SBRT, have presented a handbook on safe practice guidelines on SBRT treatment.

The consensus indications and contraindications for postoperative spine SBRT are (Table 18):

Table 18 Consensus indications and contraindications. Adapted from [84].

For postoperative spine SBRT	
Indications	Have 1-2 primary radioresistant levels of adjacent disease;
	Previously performed radiotherapy.
Contraindications	Have more than three contiguous vertebral bodies;
	Features complete spinal cord injury without preservation of motor or sensory function, i.e., ASIA Grade A status.
	Features spinal cord compression without any cerebrospinal fluid (CSF) around the spinal cord, i.e., postoperative Blisky Grade 3 residual.

Concerning the delineation of the target volume, most experts agreed on the regions to be included in the design of the structures. For target volume delineation, in preoperative cases MRI is performed and in postoperative cases T1-weighted MRI (obtained with and/or without gadolinium) to the CT simulation is obtained. For spinal cord delineation/thecal sac and paraspinal disease extension, T2-weighted MRI or

CT myelogram in cases of significant hardware artefacts are similarly recorded [60, 84].

Table 19 summarizes the structures that should be included in each type of volume or structure for a correct treatment planning.

Table 19 Consensus and predominant practices for the delineation tumor volume for postoperative spine SBRT. Adapted from [84].

Volume	Include
Planning target volume (PTV)	0-2mm expansion from CTV
Clinical tumor volume (CTV)	Entire extent of preoperative tumor, anatomic compartment involved, & any postoperative residual;
	Surgical instrumentation & incision not included (unless involved);
	Prophylactic circumferential treatment of epidural space controversial;
	Additional expansion up to 5 mm for paraspinal extension controversial;
	Consider an additional expansion of up to 5 mm cranio-caudally beyond known epidural disease extent based on pre- and postoperative imaging
Gross tumor volume (GTV)	Based on MRI – postoperative residual
Spinal cord	True spinal cord: postoperative T2-weighted MRI or CT myelogram (in cases of significant hardware artefact)
Spinal cord risk volume (PRV)	0-2mm expansion of spinal cord volume

Prescription doses and times of fractionation were controversial among radiation oncologists. For re-irradiation, with a previous irradiation treatment, the repair and the time interval between the previous RT and the spinal SBRT were included in the calculation of the cord constraints. The following table represents the common dose and fractionation schemes for various possible clinical situations (Table 20).

Table 20 Clinical scenario versus reasonable dose and fractions. Adapted from [84].

Clinical scenario	Reasonable dose x fractions
No prior RT; Single vertebral level; No epidural disease	16-24Gy x 1 fraction; 12Gy x 2 fractions; 8-10Gy x 3 fractions.
No prior RT; Multiple vertebral levels and/or epidural disease	16-18Gy x 1 fraction; 1Gy x 2 fractions; 8-10Gy x 3 fractions; 6-8Gy x 5 fractions.
No prior RT; Epidural disease	16-18Gy x 1 fraction; 12Gy x 2 fractions; 8Gy x 4 fractions; 6-7Gy x 5 fractions.
Prior RT; Any extent of epidural disease	16-18Gy x 1 fraction; 12Gy x 2 fractions; 7-10Gy x 3 fractions; 8-10Gy x 4 fractions; 5-7Gy x 5 fractions.

The treatment plan approved by the Radiation Therapy Oncology Group (RTOG) for the calculation of dose within a medium with heterogeneities should be used in all cases of SBRT in the postoperative column. The treatment planning system and the corresponding algorithm are shown in (Table 21).

Table 21 Treatment planning algorithms for calculation of dose approved by RTOG. Adapted from [84].

Treatment planning system	Algorithm
BrainLab	Monte Carlo
Corvus	Monte Carlo
CyberKnife Multipan	Monte Carlo
Eclipse	AAA (analytical anisotropic algorithm)
Eclipse	Acuros
Helax	Collapsed cone
In house	Monte Carlo
Monaco	Monte Carlo
Pinnacle	Collapsed cone convolution-adaptive convolve
Tomotherapy	Convolution superposition
XiO	Superposition-fast superposition

The time between initial treatment and re-treatment in determining spinal cord restrictions is controversial. On the dosimetric target, most radio-oncologists indicated a preference that had as goal 95% to 100% of the prescribed dose to cover 95% of the PTV.

Chapter 5

5. Conclusion and Future work

In this final chapter the main conclusions are presented after the elaboration of this work. An analysis of the accomplishment of the initially stipulated goals will be presented. Finally, a reference for possible future work to develop in this area of radiotherapy.

5.1 Final conclusions

Re-irradiation is a very important tool due to the increasing survival of cancer patients and the development of second tumors and recurrences. To evaluate the necessary conditions for re-irradiation it is necessary to consider several facts associated with the first treatment. Factors like the total dose or dose per fraction, the volume and the segment of the spinal cord to be irradiated and the time interval between irradiations are very important. Re-irradiation must be delivered only when the BED of the first treatment is lower than 102 Gy_2, when the time interval between irradiations is higher than 6 months, and total BED is lower than 135Gy_2.

From the results shown in the literature, we can conclude that the SBRT treatment is highly effective as it can deliver ablative doses to the target while sparing critical organs at risk. Although the studies analysed encourage us to practice this type of treatment, it is necessary to mention that the lack of follow-up of the patients in several studies is a worrying point.

The table developed by Nieder *et al*., [31] is an interesting working basis for the radiation oncologists to use on a re-treatment and thus to consider the risk of the patient developing radiation myelopathy.

Similarly, the article published by Redmond *et al*., [84] is a guide for the practice of SBRT because it concludes information regarding with patients are eligible for this type RT treatment.

5.2 Objectives achieved

The main objective of this work was to conduct an analysis of available articles dealing with the subject of "dose effects on spinal cord re-irradiation".

From this analysis a series of conclusions are put forward regarding dose, fractionation, tolerance tissues, regarding re-irradiation of spinal cord.

A review article was been submitted to AAPM Journal, Medical Physics. This article can be consulted Annex B in the section "Appendix B".

5.3 Future work

From work presented in this thesis it is clear that some issues still require further elucidation:

- Understanding the importance of the spatial distribution of dose (and, hence, the utility of partial circumferential sparing);
- More data is necessary to better estimate the risk of acute and long-term toxicity (including all information regarding history of concurrent and prior therapies, time interval from 1st treatment, dose and fractionation, and treatment-related toxicity, particularly neurologic deficits, for SBRT of spinal lesions);
- Furthermore, when adverse events do occur, it would be most helpful if the involved critical structure doses would be referred;
- Further investigation of radiosensitinzing drugs that act in synergy with the high doses of SBRT.

Appendix A

Annex A – Framework and development of the BED formula

The linear quadratic model of cell survival is used to describe the relationship between the total iso-effective dose and the dose per fraction in fractionated radiotherapy. This model can create an equilibrium environment between the acute and late reactions and the effects on the tumor, depending on the dose change per fraction and the total dose [9].

Cell death by irradiation can be expressed by [29]:

$$Cell\ death = E = n(\alpha d + \beta d^2)\ (Eq.5)$$

Where:

- α and β represent the radiosensitivity coefficients;
- n represents the number of fractions;
- d represents the dose per fraction.

Thus, the total dose (D) is defined as (Eq.6):

$$D = nd\ (Eq.6)$$

Considering the progressive reduction of d until it approaches zero, and although the number of fractions must then increase to maintain the same effect, βd^2 will be very small when compared to αd. Thus, when d has a very small value, the equation is approximated as (Eq.7) [29]:

$$E = n\,\alpha d = \alpha D\ (Eq.7)$$

Equation 7 shows that the total dose (D) at a very low dose per fraction represents the highest total dose required to obtain a specific effect. The total dose required is the definition of BED for situations where cell repopulation can be ignored (Eq. 8) [29]:

$$BED = D = \frac{E}{\alpha}\ (Eq.8)$$

The formula that considers the incomplete repair of damages is represented in (Eq. 9), where H_m is a tabulated value and represents the amount of unrepaired damage, being m the number of fractions [9]:

$$EQD_2 = D\ \frac{d(1 + H_m) + \left(\alpha/\beta\right)}{2 + \left(\alpha/\beta\right)}\ (Eq. 9)$$

The next formula (Eq. 10) considers repopulation that occurs in tumors and acute response tissues, where TP and TK are tabulated values [9]:

$$BED = nd\left[1 + \frac{d}{\left(\alpha/\beta\right)}\right] - \frac{0{,}693}{\alpha TP}(T - TK)\ (Eq. 10)$$

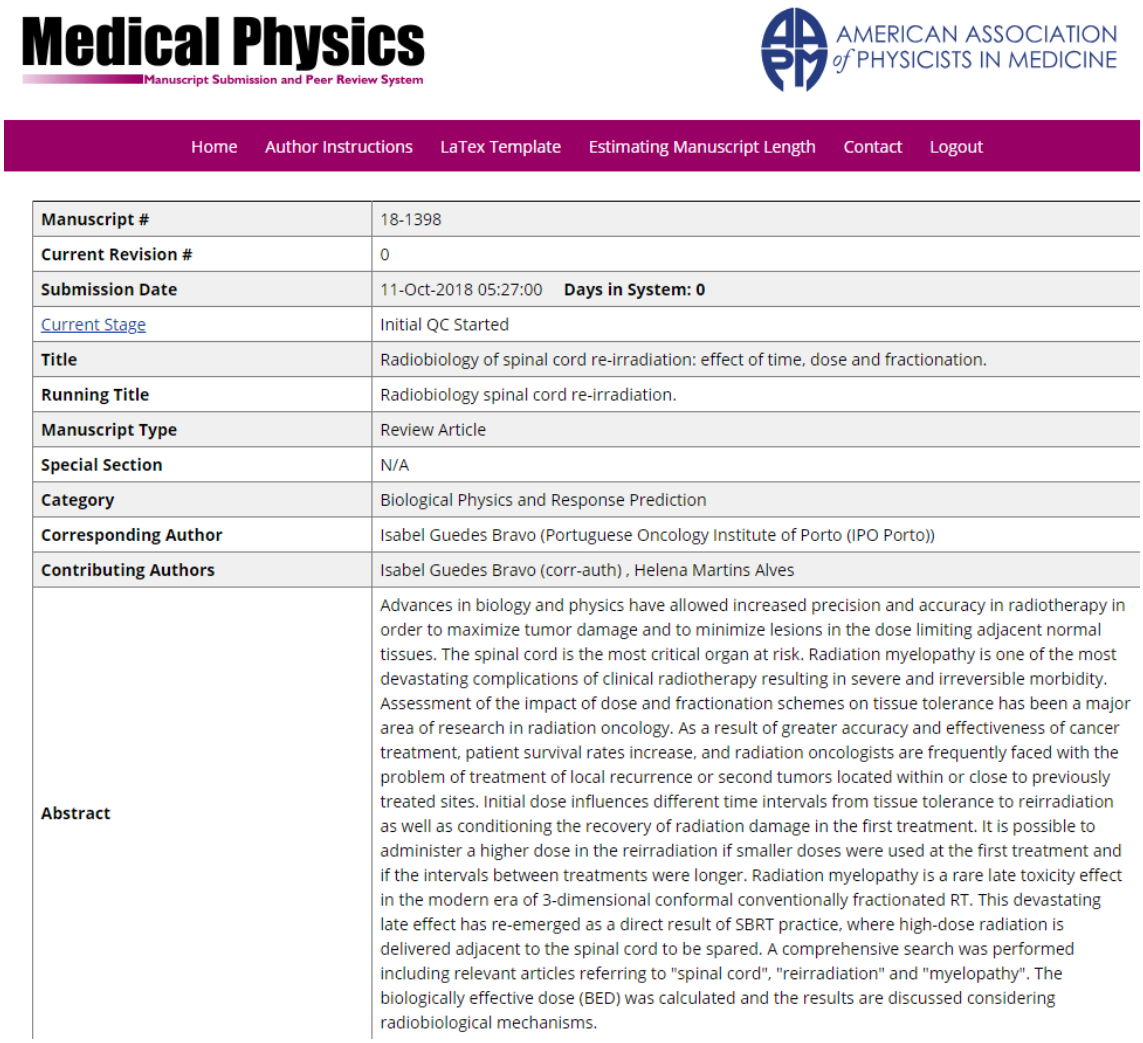
Where:

- T: Total number of days;
- TP: Cell doubling time in a tissue during radiotherapy;
- TK: Kick-off time: the apparent starting time of rapid compensatory repopulation in tumor or tissue after the start of treatment.

Appendix B

Annex B – Submission of a review article to Medical Physics AAPM

In October 2018, I submitted a review article in the journal "The International Journal of Medical Physics Research and Practice" belonging to the American Association of Physicists in Medicine (AAPM). Figure 21 is a proof.



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Manuscript #	18-1398
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Title	Radiobiology of spinal cord re-irradiation: effect of time, dose and fractionation.
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Manuscript Type	Review Article
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Category	Biological Physics and Response Prediction
Corresponding Author	Isabel Guedes Bravo (Portuguese Oncology Institute of Porto (IPO Porto))
Contributing Authors	Isabel Guedes Bravo (corr-auth) , Helena Martins Alves
Abstract	Advances in biology and physics have allowed increased precision and accuracy in radiotherapy in order to maximize tumor damage and to minimize lesions in the dose limiting adjacent normal tissues. The spinal cord is the most critical organ at risk. Radiation myelopathy is one of the most devastating complications of clinical radiotherapy resulting in severe and irreversible morbidity. Assessment of the impact of dose and fractionation schemes on tissue tolerance has been a major area of research in radiation oncology. As a result of greater accuracy and effectiveness of cancer treatment, patient survival rates increase, and radiation oncologists are frequently faced with the problem of treatment of local recurrence or second tumors located within or close to previously treated sites. Initial dose influences different time intervals from tissue tolerance to reirradiation as well as conditioning the recovery of radiation damage in the first treatment. It is possible to administer a higher dose in the reirradiation if smaller doses were used at the first treatment and if the intervals between treatments were longer. Radiation myelopathy is a rare late toxicity effect in the modern era of 3-dimensional conformal conventionally fractionated RT. This devastating late effect has re-emerged as a direct result of SBRT practice, where high-dose radiation is delivered adjacent to the spinal cord to be spared. A comprehensive search was performed including relevant articles referring to "spinal cord", "reirradiation" and "myelopathy". The biologically effective dose (BED) was calculated and the results are discussed considering radiobiological mechanisms.

Figure 21 Review article submitted to "The International Journal of Medical Physics Research and Practice".

A copy of the article:

Radiobiology of spinal cord re-irradiation: effect of time, dose and fractionation

Abstract: Advances in biology and physics have allowed increased precision and accuracy in radiotherapy in order to maximize tumor damage and to minimize lesions in the dose limiting adjacent normal tissues. The spinal cord is the most critical organ at risk. Radiation myelopathy is one of the most devastating complications of clinical radiotherapy resulting in severe and irreversible morbidity. Assessment of the impact of dose and fractionation schemes on tissue tolerance has been a major area of research in radiation oncology. As a result of greater accuracy and effectiveness of cancer treatment, patient survival rates increase, and radiation oncologists are frequently faced with the problem of treatment of local recurrence or second tumors located within or close to previously treated sites. Initial dose influences different time intervals from tissue tolerance to re-irradiation as well as conditioning the recovery of radiation damage in the first treatment. It is possible to administer a higher dose in the re-irradiation if smaller doses were used at the first treatment and if the intervals between treatments were longer. Radiation myelopathy is a rare late toxicity effect in the modern era of 3-dimensional conformal conventionally fractionated RT. This devastating late effect has re-emerged as a direct result of SBRT practice, where high-dose radiation is delivered adjacent to the spinal cord to be spared. A comprehensive search was performed including relevant articles referring to “spinal cord”, “re-irradiation” and “myelopathy”. The biologically effective dose (BED) was calculated and the results are discussed considering radiobiological mechanisms.

Keywords: *Spinal cord, Radiobiology, Re-irradiation, Tolerance.*

Introduction

Advances in biology and physics have allowed increased precision and accuracy in radiotherapy in order to maximize tumor damage and to minimize lesions in the dose limiting adjacent normal

tissues. This led to an evolution in the treatment of tumors and consequently survival rates have been increasing for a wide variety of malignancies.

The dose of radiation that can be delivered for tumor control is limited by late and generally irreversible injury of the surrounding normal tissues and organs at risk (OAR), also known as late effects.

The spinal cord is the most critical organ at risk. It is typically located near the vertebral tumor target volume and has been classically described as an organ with a serial functional architecture and as such damage to small volumes within the structure can have a major impact on neurologic function [(1)].

Spinal cord thus limits the dose to tumors in the head and neck, thoracic and upper abdominal regions as well as in cases of bone metastasis in the spine (this is estimated to occur in about 40% of cancer patients) [(2)].

As a result of greater accuracy and effectiveness of cancer treatment, patient survival rates increase, and radiation oncologists are frequently faced with the problem of treatment of local recurrence or second tumors located within or close to previously treated sites [(3)].

Spinal cord doses and tolerance

Radiation myelopathy is one of the most devastating complications of clinical radiotherapy resulting in severe and irreversible morbidity. Assessment of the impact of dose and fractionation schemes on tissue tolerance has been a major area of research in radiation oncology.

A comprehensive set of dose tolerance limits for normal tissue to radiation therapy (RT) became a reference landmark in radiation oncology [(4)]. In an extensive review of spinal cord re-irradiation it was concluded that a dose of 50Gy in the spinal cord causes a risk of myelopathy of 0.2%. If the dose increases to 59Gy the risk of myelopathy increases to 5% [(5, 6)].

There is consensus regarding the dose of tolerance accepted in the spinal cord that with conventional fractionation of 2Gy per day including the full cord cross-section, a total dose of 50Gy, 60Gy and 69Gy is associated with a 0.2, 6 and 50% rate of myelopathy [7].

As a result, standard RT involving spinal cord treatment delivers a maximum dose of 50Gy with conventional fractionation 1.8-2Gy per fraction. Data obtained experimentally indicates that dose reduction per fraction below 2Gy does not significantly alter the absolute dose-response [5].

Dose and fractionation

The biological effects of a physical dose depend on the radiobiological features of the tissue, total dose and fractionation scheme, as well as total treatment time.

The biologically effective dose (BED) is the parameter which quantifies the overall biological effect on a given tissue. The equation for BED provides a simple and straightforward way to compare doses from different fractionation schemes, which in turn have different biological effects [8, 9, 10].

The biologically effective dose indicates the radiosensitivity of normal or tumor cells to the effect of radiation. Using BED, it is possible to overcome the difficulty in clinical practice of calculating the total dose when a change in dose per fraction occurs: $BED = n \times d \times [1 + d\left(\alpha/\beta\right)]$, where n represents the number of fractions and d represents the dose per fraction, with α/β representing the sensitivity of the tissue.

For acute response tissues the α/β ratio is within a range of 7-20Gy, whereas for late response tissues the α/β ratio generally ranges from 0.5-6Gy. The increase in BED values is higher in tissues with low α/β ratios, i.e., it is higher in late response tissues than in acute response tissues [9,11].

The $^{\alpha}/_{\beta}$ values for cervical and thoracic cord are 2Gy or 4Gy, respectively. In this way, a dose of 50Gy given in 2Gy daily fractions is equivalent to a BED of $100Gy_2$ ($^{\alpha}/_{\beta} = 2$) or $75Gy_4$ ($^{\alpha}/_{\beta} = 4$).

Based on experimental and theoretical considerations, this model is mechanically plausible for designing protocols in the dose range up to 10Gy / fraction, and, based on animal data, it is reasonable up to 15 to 18Gy per fraction [12].

For a total dose of 34Gy to the spinal cord, it is estimated that after 1 year recovery is 76%. For a total dose of 38Gy it is estimated that after 2 years, a recovery of 85% occurs [2].

Initial dose influences different time intervals from tissue tolerance to re-irradiation as well as conditioning the recovery of radiation damage in the first treatment. It is possible to administer a higher dose in the re-irradiation if smaller doses were used at the first treatment and if the intervals between treatments were longer [3, 13].

When assessing tumor re-irradiation, it is necessary to consider whether the dose of tolerance of an organ has already been reached in the first course of treatment. Treating an area that had prior radiation is far more complex than the initial treatment.

Key re-irradiation parameters evaluated in the decision process include [13, 14]:

- Data from previous treatment such as irradiated spinal cord volume and region;
- Total dose;
- Dose per fraction administered;
- Time elapsed since the first treatment.

Time interval between fractions / total treatment time

Re-treatment in periods shorter than two years may increase the risk of developing complications, since lesions are more likely to develop during this time period [5]. Nevertheless, when the time interval between the first treatment and the re-treatment is less than 6 months; the biologically

effective dose of each treatment is less of $98Gy_2$, no cases of radiation myelopathy where observed when cumulative BED is $\leq 120Gy_2$ [10].

Experimental evidence from literature corroborates that the capacity of recovery spinal cord recovery depends on the time interval between the two treatments. Although there is evidence supporting the recovery of the spinal cord, there are still some controversies in the translation of experimental animal data to clinical situations.

In schemes with multiple daily fractions it is essential to create an interval between fractions as long as possible. Due to the catastrophic effect of radiogenic myelopathy repair of sublethal damage in the spinal cord may not be complete after an 8 hour interval [3]. Repair kinetics of the spinal cord is slower, so it is not advantageous to use schemes with time intervals of less than one day.

Significant recovery of long-term damage occurs in rat spinal cord [13]. Organ tolerance to re-irradiation is influenced by the level of initial damage and time of onset of myelopathy (latency time). Thus, increasing time interval between treatments results in greater recovery and, consequently to lower doses delivered in re-treatment [2, 3, 13].

Time of recovery from damage depends on the tissue or species, as well as on the age of the individual: In animal experiments performed it was observed that 3-week-old mice showed effects faster than adult mice. However, long-term recovery occurred more rapidly in younger rats (3 weeks old) [2, 3].

Long-term recovery was observed after 8 weeks and it increased with time interval to re-treatment. The initial dose influenced tolerance to re-treatment and radiation damage repair at different intervals with long-term recovery after 6 months being approximately 45% [3, 13].

Pathophisiology

Radiation injury to the spinal cord is among the most studied experimental models of radiation injury and probably is the most fully documented clinical radiation complication.

To the thoracic cord, it is still apparent that its sensitivity to radiation is substantially less than that of the cervical cord. A significant oxygen effect exists, and it is possible that the thoracic cord is intrinsically less well oxygenated. Extrinsic conditions, such as smoking history or effects of lung cancer may result in the patients with thoracic irradiation having less well oxygenated nervous tissues. Haemoglobin levels are significantly greater in thoracic patients with myelopathy that in those without myelopathy treated to the same dose [(15)].

The incidence of cervical myelopathy at 45Gy is, approximately, 0.03% and at 50Gy is, approximately 0.2%. Large lengths of cord, concomitant chemotherapy and other factors may increase this incidence [(15)].

Spinal cord, besides being one of the most dose-limiting organs in radiotherapy, is also a risk organ present in several neoplasms. It is a serial architectural organ, being very sensitive to radiation in small portions of its extension. There is a possibility that the sensitivity of the spinal cord depends on the organization and structural distribution of its functional subunits (FSU), since there are structural differences between the different spinal segments. These differences are mainly patented in grey and white matter [(16, 17)].

The effects arising from the volume of irradiated bone marrow may be related to vascular supply, collateral circulation and/or the ability to re-establish damaged vasculature through revascularization. There is also the issue of the release of cytokines and mediators of inflammation. Their release may be affected by the irradiated volume because the larger the volume the greater the release of these potentially harmful substances [(5)].

In summary, recovery from initial damage is well established. The amount of recovery seems to be influenced by different factors, with initial damage expressed as a percentage of cord tolerance being the most important one. Time interval between the first treatment and re-treatment is also important as it is estimated than % recovery.

The real total tolerance of the spinal cord may be about 130% if the tolerance dose for the first treatment as estimated by Kogel [(18)]. Although the general phenomenon of repair occurs in humans

caution is necessary when translating experimental animal data to the clinical setting because of the life span, latency and dose response difference [19]:

- Histological pattern of myelopathy in animals differs from those found in humans;
- Repair kinetics is probably different, and the time to achieve maximal repair and re-tolerance in humans [18]. It is of extreme interest to consider the knowledge about the kinetics of the evolution of the hidden damages caused by the previous treatment.

In general, a higher re-treatment dose can be given following lower initial doses and longer intervals between treatments. From the sparse clinical and primate data, it appears that at least 50% recovery from 45Gy would be obtained 2 years after treatment.

Spine Stereotactic Body Radiation Therapy (SBRT)

Spine SBRT, also known as spine stereotactic radiosurgery (SRS), is an emerging treatment option for patients with spinal bone metastases with or without a soft tissue component and is rapidly being adopted in the clinical although with limited high-quality evidence [1, 20].

SBRT is a highly effective treatment able to deliver ablative doses to tumors with minimal doses to the surrounding normal tissues. It Is defined as radiotherapy treatment of tumors outside the brain with 1 to 5 dose fractions. It has been made possible in recent years due to advances in image guidance, target visualization and radiation delivery methods that allow large doses delivered to the target while sparing critical organs at risk (OARs).

Outstanding results already have led to the suggestion that high doses per fraction may have greater efficacy than conventional fractionated radiation therapy [21]. Local control rates in spine re-irradiation with SBRT reported in the literature range from 66% to 92% at 1 year [22].

Initially, this technique was used to successfully palliate spinal metastases, even for tumors of radio-resistant histologies. This success with spinal metastases has led to the widespread extrapolation of SRS to the treatment of primary spine tumors and for benign tumors of the spinal cord [1, 23].

SBRT has specific characteristics that differentiate it from conventional RT [24, 25]:

- *Treatment scheme*, where dose delivery is performed in one or a few fractions, resulting in a high BED value. In several studies, we can verify that a re-irradiation performed with a SBRT treatment has higher BED values when compared to the first conventional radiotherapy treatment (Table 1).
- *Small irradiated volumes;*
- *Inhomogeneous dose distribution* (the inherent sensitivity of the dose distribution secondary to the steep dose gradient beyond the target volume);
- *Short treatment time* (in a conventional treatment since the duration of the treatment would last for several months).

In the limits defined by Emami [4, 6] all prescriptions were in 1.8 or 2Gy fractions. In contrast, SBRT prescription schemes typically range from 5Gy per fraction up to 20Gy per fraction or more. In this new radiation delivery paradigm, normal organ dose tolerance limits and the dose-volume response of the tumors depend strongly on the number of fractions used and the dose per fraction.

After more than 20 years of Radiation Therapy Oncology Group (RTOG) trials, conventional radiation therapy had progressed to the point where it is possible to define dose tolerance limits in terms of 5% or 50% chance of a specified adverse event occurring within five years. In contrast, published follow-up data for SBRT is inadequate to reliably determine the probability of adverse events [29].

Radiation myelopathy is a rare late toxicity effect in the modern era of 3-dimensional conformal conventionally fractionated RT. This devastating late effect has re-emerged as a direct result of SBRT practice, where high-dose radiation is delivered adjacent to the spinal cord to be spared. Possible reasons include [30]:

i. Lack of understanding of tolerance to the spinal cord with high dose per fraction radiation therapy;

ii. Sensitivity of the spinal cord in SBRT dose distribution to small intrafraction motions where even 1- to 2mm shifts during treatment can increase the actual spinal cord dose delivered;

iii. An assumption that a small point volume within the spinal cord can tolerate a higher dose of radiation than would traditionally be considered safe.

Radiobiology: impact of repair, redistribution, repopulation, reoxygenation and radiosensitivity (5Rs)

The classical fractionation principles, i.e., the Rs of radiobiology, explain the effects of high doses of ionizing radiation on tumors and adjacent normal tissues (Table 2). The outcome of standard clinical radiation treatment is determined by the Rs of radiobiology:

Repair of sublethal DNA damage: normal cell are more effective than tumor cells in this process as observed from cell recovery in the 2 hour period after exposure to ionizing radiation. Radiation randomly interacts with molecules in the cell, but DNA is the main target molecule for the biological effects of radiation, including cell killing, carcinogenesis and mutation. In radiotherapy radiation damage is primarily manifested by the loss of reproductive capacity. Radiation causes a wide range of lesions in DNA such as single (SSB) and double-strand breaks (DSB) in the sugar-phosphate backbone of the DNA molecule. SSB can be readily repaired using the undamaged chromatid as a template. The most deleterious lesion induced by ionizing radiation is DSB, a break in both strands of 10 base pairs or less. There are several mechanisms to repair DSBs, which indicate the importance and difficulty of repairing this type of DNA injury. The most important are non-homologous end joining (NHEJ) and homologous recombination (HR). Homologous recombination provides greater repair accuracy than NHEJ, the major pathway to repair DSB throughout all the phases of the cell cycle [(34)].

Redistribution: when radiotherapy is given to a heterogenous cell population, cells may be in different phases of the cell cycle. Cells in S phase are more radioresistant and cells in late G2 and M phases are more sensitive. A small dose of radiation will destroy the more sensitive cells, and a resistant cell population that is now synchronized survives. As fractionated radiotherapy

treatment continues, the resistant surviving cells will continue throughout the cell cycle and when a new dose is delivered some of these cells have moved from a resistant to a more sensitive stage and will then be killed more easily [35].

Repopulation: Some time after irradiation an increase in cell division is seen in normal and malignant cells. Repopulation occurs at different rates depending on the tissues and represents cell proliferation that aims at compensating the cell population that was killed.

This homeostatic response to cell loss occurs in situations other than irradiation and is related to specific cell-cycle time: as a result of radiotherapy cell death occurs after irradiated cells attempt mitosis and thus highly proliferative tissues (and tumors) show damage much faster than slowly proliferative tissues.

Reoxigenation: The level of oxygenation in a tumor is a major determinant of the effectiveness of radiotherapy. Tumor cell microenvironment presents areas with decreased pH, lack of nutrients and hypoxia. Oxygen concentration (pO2) varies between 10- and 80-mm Hg in normal tissues but in tumors these values can be lower than 5 mm Hg in some areas. This anomaly is due to the development of abnormal vasculature during tumor angiogenesis. A significant proportion of tumor cells is hypoxic, showing great heterogeneity that is not correlated with standard prognostic factors such as size, stage and histological type [36].

Hypoxia may have a crucial role in treatment outcome and may also influence metastatic capacity of tumor cells resulting from genetic changes such as those involving blood oxygen transport or inducing vascularization. As a result of prolonged exposure to hypoxia, cells can acquire genetic resistance to apoptosis suggesting that hypoxia can favour tumor progression through clonal selection of cells with more aggressive phenotypes [37].

After an initial dose of radiotherapy, the more sensitive oxygenated cells are killed; during reoxygenation, surviving tumor hypoxic cells can increase their oxygen supply thus increasing their sensitivity to radiation. Biological efficacy of ionizing radiation relies on oxygen interacting with cells and making DNA lesions permanent [38].

Thus, tumor hypoxia may actually be a more serious problem with SBRT due to a reduction in reoxygenation between fractions as associated with small number of SBRT fractions (1 to 5).in comparison with conventional RT. This problem could be overcome if the next fraction of SBRT could be to delivered after tumor reoxygenation. And although there are no standard intervals between the high doses of SBRT, good clinical results were obtained in spite of different fractionation intervals [39].

Dividing total dose in several fractions saves normal tissue due to sublethal damage repair and cell repopulation occurring between treatment fractions. At the same time fractionation allows greater damage to the tumor as a result of reoxygenation of hypoxic cells as well as redistribution of cells into more radiosensitive stages of the cell cycle. Therefore, DNA repair and cell repopulation mechanisms induce normal tissues to become more resistant to a following dose of radiation; the other Rs, namely redistribution and reoxygenation have the opposite effect increasing radiosensitivity of tumor cells. The Rs represent the factors that modify tissue response to fractionated radiotherapy - overall radiosensitivity of a specific tumor depends upon a 5th R: Radiosensitivity [40], that translates the outcome of all the other Rs and represents individual sensitivity to radiation. These five fundamental factors represent the biological basis of radiotherapy.

Some radiobiological questions remain without response regarding the evaluation of the doses administered in the SBRT and the effect of high doses per fraction. While in conventional radiotherapy the treatment time is approximately 5 minutes, in the SBRT the treatment session takes most longer (15-40 minutes).

The major question raised when applying these radiobiological principles to SBRT is whether the fractionated dose of radiation administered in daily intervals is equivalent to the few large doses administered without breaks (as repair of sublethal damage occurs within the time interval between the administration of two doses of radiation in conventional fractionation) [32, 41].

As SBRT uses doses per fraction much higher than conventional radiotherapy, the radiobiological mechanisms underlying radiation response have been subject to controversy, regarding their application to extreme hypofractionation [41]. Hypofractionation does not follow the basic principles of radiobiology to explain tumor responses obtained by conventional fractionation schemes. Thus, the relevance of the Rs of radiobiology in a scenario of hypofractionation is controversial: As the treatment time decreases, repopulation will be of lesser importance and the clinical significance of cell redistribution in the cell cycle, in a scenario of hypofractionation, is unclear. The advantages and disadvantages of the radiobiological mechanisms are presented in table 3.

In spite of the controversy around the relevance of the 5Rs in SBRT, [32,42], it is now clear that these radiobiological concepts are sufficient to explain the impressive results obtained from clinical studies are the result of much larger biologically effective doses delivered [43].

Table 4 presents information regarding 1st treatment and re-irradiation data from several studies. Also included are values regarding:

- Total dose and the number of fractions delivered, both in the initial treatment and in the re-treatment;
- Time elapsed between the end of the first treatment and the re-treatment;
- α/β ratio;
- Treatment area (cervical, thoracic, lumbar and sacral);
- Follow-up time;
- Time until radiation myelopathy appears, should it occur.

Based on the classification developed by Nieder *et al*., [10] (Table 4): when the interval between the two treatment courses is not shorter than 6 months and the dose of each course is BED $\leq 98 Gy_2$, the cumulative BED where no case of radiation myelopathy (RM) has been reported is $120 Gy_2$; for values between 120 and 135 Gy_2, the risk of RM is small.

Analyzing table 5 it is possible to highlight the following:

- Data presented by Wong *et al*., [44]: patient received 20.6Gy in the 1st treatment in 6 fractions and was retreated after 4 months.

  BED for 2nd treatment was 102.65Gy_2; cumulative BED was 158.58Gy_2. His risk score was 4, wich presented an intermediate risk of developing myelopathy, which occurred after 6 months;

- Data presented by Schiff *et al*., [45], Grosu *et al*., [26], Nieder *et al*., [10] and Maranzano *et al*., [47]: the cases reported had a time interval between treatments of more than 6 months, BED is $\leq$ 98Gy_2 for each treatment, cumulative BED=120Gy_2 and no cases of myelopathy;

- Data presented by Sahgal *et al*., [28]: patient had a time interval between treatments of 5 months; cumulative BED=204.4Gy_2 and a high risk of developing myelopathy (score of 9). The follow-up time was very short (4 months) for the manifestation of symptoms, should they occur.

- Data presented by Sahgal *et al*., [30]: 9 cases of patients developing myelopathy were reported. BED values ranged from 181.3 to 337.5 for the re-treatment. Time to manifestation of symptoms in this group of patients ranged from 3 to 15 months.

Limitations and constraints

Most studies related to re-irradiation are based on data from retrospective studies or small prospective studies. In addition to this, reirradiated populations are more heterogeneous than patients with local or regional recurrence or second tumors [48].

The major paradigm associated with spinal cord re-irradiation is related to the lack of evidence regarding repair of long-term damage in this organ. There is a greater uncertainty related to

tolerance rates during re-irradiation, since most of the existing data are derived from results obtained with animals, revealing long-term damage recovery [13].

As SBRT is increasingly being employed in the treatment of spinal lesions, and although reports of toxicity are rare, the follow-up time is short and patient numbers are still small. The effect of concurrent chemotherapy is essentially unknown and needs to be investigated.

The consensus is that SBRT is appropriate in the postoperative cases in patients with limited disease, radioresistant tumors and as another treatment strategy after failure of conventional radiotherapy treatment.

The optimal dose prescription for SBRT remains controversial. On the one hand, a single fraction treatment results in better local control rates than obtained with conventional RT. This may be the result of larger biologically effective doses delivered with SBRT. Alternatively, higher rates of local control following single fraction treatment in retrospective series may simply reflect better outcomes in patients with less aggressive disease [49].

Table 22 Variation in BED value according to treatment schedule.

		Initial treatment		**Re-irradiation treatment**	
Author	α/β value	Dose fx(Gy)/no fx	BED	Dose fx(Gy)/no fx	BED
Grosu *et al*., (26)	2	1.25/32	65.0	1.8/16	55.1
		1.4/20	49.4	3.0/10	75.0
Wright *et al*., (27)	4	3.0/10	53.0	6/5	26.0
	4	3.0/15	79.0	6/5	15.0
	2	3.0/11	83.0	6/5	20.0
Sahgal *et al*., (29)	2	1.8/30	102.6	8.0/3	120.0
		0.9/28	36.5	10.5/2	131.3
		2.88/15	105.4	16.0/1	144.0

Table 23 Impact of radiobiological mechanisms in SBRT treatment.

Biological mechanisms	Description
Repair of sublethal damage	Treatment with SBRT has a longer irradiation time when compared to conventional radiotherapy. With this treatment, repair of sub-lethal damage may occur during prolonged exposure to radiation. After 30 minutes, the sublethal damage repair is greater than >10% [31, 32].
Repopulation of cells after radiation	Repopulation occurs depending on the type of tissue and the dose of radiation administered. In conventional radiotherapy, tumor cell repopulation occurs within 2 to 3 weeks after start of RT. Thus, it is possible that the repopulation of tumor cells may occur earlier in the treatment of SBRT or SRS than in conventional RT [32]. Delivering a higher dose should mitigate any clonal expansion and thus offer a significant advantage to rapidly dividing tumors [33]. As treatment time of SBRT lasts is short, 2 weeks at most, repopulation of tumor cells will not be a problem [31].
Redistribution of cells within the cell cycle	Reducing the number of fractions alters the probability of irradiating a cohort of cells as they move into a radiosensitive phase [33]. After irradiation with a single fraction of 15–20Gy, cells are indefinitely arrested in the phases of cell cycle where they were irradiated and undergo interphase death [31]. After irradiating the cell cycle with a dose of 20Gy in a single exposure, cell cycle progression was delayed, and many cells underwent necrosis in the phases of the cycle where they were irradiated. The cells that were irradiated slowly progressed to the G2 phase and died [32].
Reoxygenation of the surviving cells	In SBRT, due to the vascular damage caused by the high doses of radiation, the intratumoral environment becomes hypoxic. Hypoxic cells reoxygenated when irradiated with a relatively low dose per fraction, i.e., less than 10Gy [31]. Following administration of a SBRT or SRS treatment, massive vascular destruction of the tumor occurs, and reoxygenation of hypoxic cells occurs 2-3 days after irradiation. As oxygen consumption declined dramatically after massive death of tumor cells, surviving hypoxic cells can be reoxygenated. Changes in the oxygenation status of the tumors after irradiation with high doses are still unclear [32]. SBRT has a theoretical disadvantage because of the short delivery time. Although this might be compensated by the high doses per fraction.
Radiosensitivity	SBRT attenuates differences in tumor death that are directly attributable to variations in radiation sensitivity of individual tumor cells. Large doses per fraction and short treatment time used in SBRT provides less opportunity for the development of resistant stem cells [33].

Table 24 Advantages and disadvantages of radiobiological mechanisms Adapted from (33):

Radiobiological mechanisms	Advantage	Disadvantage
Repair	Improvements in the technique of dose delivery allows dose reduction delivered to adjacent normal tissues.	Limitation of the number of cycles of damage and repair that separate the tumor response from normal tissue toxicity.
Repopulation	Tumor repopulation is greatly reduced or eliminated during a treatment with less fractions. It is specifically relevant for radiation-resistant tumor stem cells.	None
Redistribution	The distribution of the cells will be affected by the reduced number of fractions. The clinical significance is still unknown, so it is not possible to enumerate the advantages and disadvantages of this biological mechanism.	
Reoxygenation	None	A smaller number of fractions potentially reduces reoxygenation between fractions, which results in an increase in radioresistance.
Radiosensitivity	A single fraction dose greater than 10Gy may trigger apoptosis of endothelial cells.	Normal late-reacting tissues are more radiosensitive at higher doses per fraction and so the risk of late complications is higher at higher doses per fraction [25].

Table 4 Risk of developing myelopathy from the characteristics mentioned in the table. Adapted from (10).

Points	Cumulative BED in Gy_2 with interval < 6 months and a BED of 1 course ≥102 Gy_2	Risk of developing myelopathy
0	≤ 120.0	Low risk (≤3 points)
1	120.1 – 130.0	
2	130.1 – 140.0	
3	140.1 – 150.0	
4	150.1 – 160.0 x (4.5) x (4.5)	Intermediate risk (4-6 points)
5	160.1 – 170.0	
6	170.1 – 180.0	
7	180.1 – 190.0	High risk (>6 points)
8	190.1 – 200.0	
9	> 200.0	

Table 5 Summary of published reports of treatments performed using re-irradiation.

		1st treatment			Re-irradiation								
Author	Sample	Total dose (Gy)	Dose fx(Gy)/no fx	Interval (months)	Total dose (Gy)	Dose fx(Gy)/no fx	Nivel treated	Value of α/β (Gy)	Initial BED	Irradiation BED	Total BED	Follow-up (months)	Time to myelopathy (months)
Wong *et al.*, (44)	11	2.0+45.9	2.0/1+3.06/15	12	23.6	2.36/10	T	2	120.13	51.45	171.58	≥60	4
		23.5+18.2	2.35/10+3.64/5	19	13.6	2.72/5	T		102.44	32.096	134.54		16
		20.6	3.43/6	4	36.4	3.64/10	T		55.93	102.65	158.58		6
		20.1	1.26/16	48	14.3+3.2 +22.6+2.57	2.38/6+1.6/2+ 3.76/6+2.57/1	T		33.25	108.04	141.29		11
		16.0	3.2/5	57	22.0	7.33/3	T		41.60	102.63	144.23		7
		24.0+24.0	3.0/8+1.5/16	45	18.1	1.81/10	T		102.00	34.48	136.48		13
		20.4+20.3	4.08/5+4.06/5	10	8.2	8.2/1	T		123.53	41.82	165.35		4
		24.4	4.88/5	2	17.0	3.4/5	T		83.94	45.9	129.84		14
		31.2	1.56/20	71	30.3	5.05/6	C		55.54	106.81	162.35		25
		33.9	3.39/10	2	25.6	2.56/10	T		91.36	58.9	150.4		13
		50.0	2.5/20	27	10.4	1.04/10	T		112.50	15.81	128.31		11
Schiff *et al.*, (45)	54	30.0	3.0/10	Median 9.1 (range: 1-51.3)	30.6	1.8/17	T	3	60.0	48.96	108.96	4.2	No cases
		30.0	3.3/9		39.6	1.8/22	L		63.0	63.36	126.36		
		30.0	3.0/10		22.0	2.0/11	C/T		60.0	36.7	96.7		
Sminia *et al.*, (46)	8	16.0	8.0/2	52	20.0	4.0/5	T	2	80.0	60.0	140.0	1-54	No cases
		39.0	3.0/13	12	21.0	3.0/7	T		97.5	52.2	149.7		
		8.0	8.0/1	4	18.0	3.0/6	C		40.0	45.0	125.0		
		49.6	1.6/31	61	50.0	2.0/25	L		69.4	75.0	144.4		
		37.4	2.2/17	30	21.0	3.0/7	T		78.5	52.2	130.7		
		24.0	3.0/8	20	21.0	3.0/7	T		60.0	52.2	164.4		
		25.0	1.7/15	150	16.0	4.0/4	T		46.0	48.0	142.0		
		35.25	1.5/23.5	73	30.0	3.0/10	L		48.5	52.5	125.0		
Grosu *et al.*, (26)	8	40.0	1.25/32	Median 30 (range: 6-63)	29.0	1.8/16	T	2	65.0	55.1	120.1	Median 16 (range: 5-44)	No cases
		30.0	3.0/10		30.0	2.0/15	L		75.0	60.0	135.0		
		40.0	2.5/16		30.6	1.8/17	T/L		90.0	58.1	148.1		
		40.0	2.0/20		30.6	1.8/17	T		80.0	58.1	138.1		
		50.0	2.0/25		18.0	3.0/6	T		100.0	45.0	145.0		
		30.0	3.0/10		30.0	2.0/15	T		75.0	60.0	135.0		
		29.0	1.4/20		30.0	3.0/10	T		49.4	75.0	124.4		
		36.0	3.0/12		30.0	2.0/15	L		90.0	60.0	150.0		
Wright. *et al.*, (27)	37	40.0	2.67/15	Median 19 (2-125)	20.0	4.0/5	SC	2	93.0	11.0	104.0	8 (range 1-51)	No cases
		30.0	3.0/10		20.0	4.0/5	CE	4	53.0	4.0	57.0		
		30.0	3.0/10		20.0	4.0/5	SC	2	75.0	17.0	92.0		
		50.4	1.8/28		20.0	4.0/5	CE	4	73.0	9.0	82.0		
		37.7	2.51/15		20.0	4.0/5	SC	2	85.0	20.0	105.0		

T, thoracic; C, cervical; L, lumbar; SC, spinal cord; CE, cauda equina. BED is calculated using the formula: $BED = n \times d \times [1 + d\left(\alpha/\beta\right)]$.

Table 5 (cont.) Summary of published reports of treatments performed using re-irradiation.

		1st treatment			Re-irradiation								
Author	Sample	Total dose (Gy)	Dose fx(Gy)/no fx	Interval (months)	Total dose (Gy)	Dose fx(Gy)/no fx	Nivel treated	Value of α/β (Gy)	Initial BED	Irradiation BED	Total BED	Follow-up (months)	Time to myelopathy (months)
Nieder *et al.*, (10)	7	60.0	2.0/30	37	64.0	2.0/32	C-T	2	37.5	70.0	107.5	7	No cases
		60.0	2.0/30	31	56.0	2.0/28	C	2	37.5	67.0	104.5	5	
		70.0	15 x 2 Gy followed by twice-daily radiotherapy with 1.8 Gy in the morning and 1.5 Gy in the afternoon	23	50.0+12.0	2.0/25+3.0/4	C	2	80.0	43.0	123.0	10	
		59.4	1.8/33	12	45.0	1.8/25	C	2	96.0	85.5	181.5	8	
		36.0	3.0/12	30	30.0	2.0/15	L-S	4	63.0	39.0	102.0	20	
		44.8	1.6/10+1.8/16	96	36.0	2.0/18	T	2	83.5	72.0	155.5	5	
		40.0	2.5/16	26	30.0	2.0/15	L	4	65.0	45.0	110.0	17	
Maranzano *et al.*, (47)	12	8.0	8.0/1	31	8.0	8.0/1	SC	2	40	40.0	80.0	SI	No cases
		8.0	8.0/1	9	16.0	2.0/8			40	80.0	120.0		
		8.0	8.0/1	9	8.0	8.0/1			40	40.0	80.0		
		8.0	8.0/1	5	8.0	8.0/1			40	40.0	80.0		
		8.0	8.0/1	2	8.0	8.0/1			40	40.0	80.0		
		8.0	8.0/1	4	20.0	5.0/4			40	60.0	120.0		
Sahgal *et al.*, (28)	19	37.5	2.5/15	5	24.0	8.0/3	C	2	84.4	120	204.4	4	No cases
		39.1	2.3/17	39	25.0	5.0/5	L		84.1	87.5	171.6	7	
		30.0	3.0/10	6	24.0	6.0/4	L		75	96	171	5	
		50.0	2.0/25	11	24.0	8.0/3	T		100	120	220	8	
		40.0	1.82/22	81	20.0	10.0/2	T		76.4	120	196.4	55	6
		25.2	0.9/28	70	21.0	10.5/2	T		36.5	131.3	167.8	29	5
		21.2	4.24/5	11	14.0	14.0/1	T		66.1	112	178.1	17	3
		51.9	1.85/28	18	33.0	11.0/3	C		99.9	214.5	314.4	11	8
		43.2	2.88/15	12	16.0	16.0/1	T		105.4	144	249.4	3	3
Sahgal *et al.*, (30)	66	NR			25.0	12.5/2	T	NR		181.3	NR	17	9
					24.0	8.0/3	C-T			120		9	9
					16.0	16.0/1	Clivus-C1			144		24	13
					25.0	25.0/1	C			337.5		40	13
					25.0	25.0/1	C			337.5		39	5
					18.0	18.0/1	T			180		24	12
					16.0	16.0/1	C			144		8	3
					14.0	14.0/1	T			112		16	6
					30.0+14.0	3.0/10+14.0/1	C			187		23	15

T, thoracic; C, cervical; L, lumbar; S, sacral; SC, spinal cord. BED is calculated using the formula: $BED = n \times d \times [1 + d\left(\alpha/\beta\right)]$. NR, not reported.

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